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Experimental evolution of ecological niches in heterogeneous environments

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Abstract

Organisms live in heterogeneous environments but fail to demonstrate optimal adaptation to the totality of the ecological conditions they experience. This imperfection would result from trade-offs between ecologically relevant traits, which implies that trade-offs are the ultimate cause of major features of life such as biodiversity. Here, I selected bacterial populations in defined laboratory environments to test for the importance of differences in productivity and quality among alternative resources (Chapter 1), as well as the spatial pattern of resources (Chapter 2), in shaping the evolution of ecological niches and genetic diversity. The experiment presented in Chapter 1 provides support for a general model for the evolution of diversity. In Chapter 2 however, I suggest that mutational processes can impinge on the expression of trade-offs, which is not recognized in our current view of the problem of diversity. These findings provide plausible explanations for situations where expected trade-offs are not found.

Résumé

Les organismes occupent des environnements hétérogènes, toutefois, ils ne sont pas adaptés à l'ensemble des conditions écologiques qu'ils rencontrent. Ce défaut résulterait de compromis entre des traits écologiquement importants, et donc que les compromis seraient à l'origine de plusieurs attributs de la nature tels que la biodiversité. Mon travail a consisté à sélectionner des populations bactériennes en laboratoire afin de tester l'importance de la productivité et la qualité de ressources (Chapitre 1), ainsi que de leur arrangement spatial (Chapitre 2), pour l'évolution des niches écologiques et de la diversité génétique. L'expérience du Chapitre 1 supporte un modèle général de l'évolution de la diversité. Dans le Chapitre 2 toutefois, je suggère que des processus mutationnels pourraient limiter l'expression des compromis, une idée qui n'est pas reconnue dans notre compréhension actuelle de la diversité. Ces résultats pourraient expliquer les situations où un compromis était attendu mais n'a pas été observé.

Remerciements

Plusieurs personnes ont fait que mes vingt mois passés à Ottawa et dans ses universités ont été stimulants. D'abord, Rees Kassen et les Études Supérieures de la Faculté des Sciences ont procédé à mon admission moins d'un mois avant le début de mon premier trimestre, en Janvier 2005. Rees a toujours été prêt à m'écouter et il a laissé libre cours à mon imagination au laboratoire, à un point tel que je fus probablement bon second après Sascha pour lui causer de l'anxiété. Je remercie Rees pour son humanisme. C'est le moins que je puisse dire.

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Introduction

The origin of life's diversity is a long standing problem in biology and a highly active field of investigation (Culotta and Pennisi 2005). In nature, individuals differ in reproductive success, therefore why is it that the earth is not inhabited by a single, best adapted competitor? This problem is known as the paradox of diversity (Hutchinson 1958; Hardin 1960) and numerous hypotheses have been proposed to solve it (Schluter and Ricklefs 1993; Silvertown 2005).

A general solution to this paradox is that biological diversity results from the diversity of the environment. The environment can be heterogeneous in many ways. For example, forests are a mosaic of shade and light gaps. Some tree species specialize at colonising light gaps, while other species are more shade tolerant. Therefore, because no tree can grow fast in light gaps and tolerate extensive periods of shade, heterogeneity in the environment promotes the coexistence of a diversity of trees (Wright et al. 2003). Central to this view of diversity are trade-offs; heterogeneous environments can only explain biological diversity if improved performance in one part of the environment comes to a cost in other parts, such that no type can reach the optimal performance under all conditions (Ackermann and Doebeli 2004). Thus, environmental heterogeneity and trade-offs are thought to be the ultimate causes for biological diversity (Ricklefs 2001).

This view of diversity is supported by the large variability found in individual performance across different patches of the environment (Bell 1997; Bell et al. 2000), indicating that the environment is heterogeneous from the point of view of organisms. Tansley (1917), for example, showed in a common-garden experiment that soil type and competition could explain the distribution and coexistence of two species of plants. He reported that although both species could grow on acidic and calcareous soils when grown alone, albeit not with the same success, only one of them survive on each soil type when both species are planted together. This specialization of types to contrasted environmental patches is quantified in a genotype-by-environment interaction in fitness, which simply means that the fitness of a given genotype depends on the environment on which it is measured. When the genotype-by-environment interaction is large (Figure 1 *A*), diversity tend to be maintained. However when the genotype-by-environment interaction is weak, such as when one genotype is superior to every other across all environmental patches (Figure 1 *B*), this genotype is predicted to take over the population, thus causing the decline of diversity.

Experimental tests of the idea that diversity is most readily maintained in heterogeneous environments require model systems in which genetic variation in fitness (i.e. variation that is heritable and has an effect on organisms' fitness) arise *de novo* and is sorted by natural selection. Experimental evolution with microbes (most often yeast, bacteria, or viruses) allows investigating evolutionary questions under these criteria. Laboratory populations of microbes can have large population sizes and short generation times, while occupying little space (billions of individuals can fit in a millilitre of liquid culture). Microbes are also easy to manipulate with molecular tools. For example, it is possible to "mark" a genotype of interest with a gene that does not influence fitness, and then to follow the fate of this genotype in a mixed population. This strategy is used extensively in these experiments to measure the fitness of novel genotypes. In summary, microbial populations allow the comparison of the appearance and maintenance of diversity over hundreds of generations in contrasted environments.

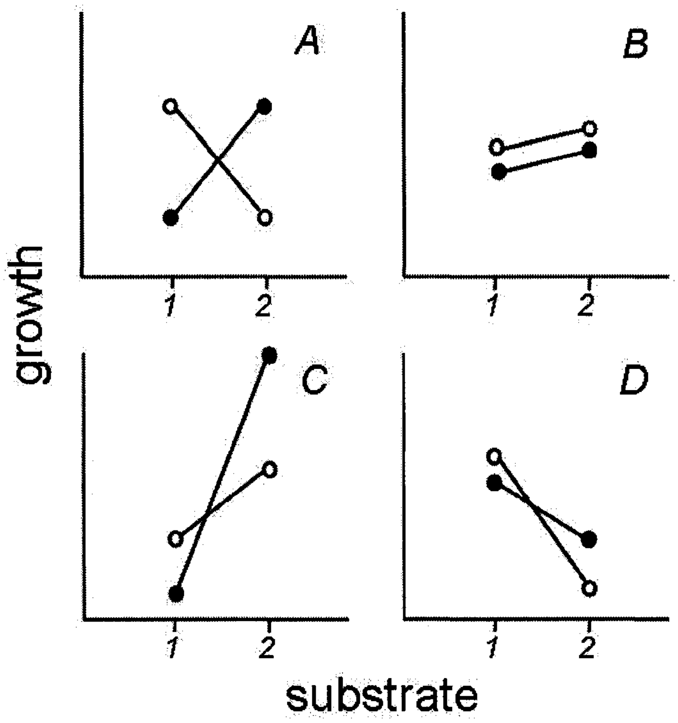

Figure 1. Growth of two individuals with distinct genotypes (filled and empty dots) on two substrates (or patch). Growth is taken as a proxy for fitness. *A*, the growth of each type is high on one substrate and low on the other, thus the genotype-by-environment interaction is strong and the genotypes will be maintained in a heterogeneous environment. *B*, one genotype grows better on both substrates and diversity will be lost. *C*, one genotype is specialized on a productive patch and may fix in the population. *D*, one genotype is specialized on the productive patch but both genotypes have similar fitness and diversity can be maintained.

The basic protocol in microbial experimental evolution is a selection experiment followed by fitness assays. This protocol was used in both chapters presented in this thesis. In this context, a selection experiment consists of growing a large clonal population of bacteria for hundreds of generations in environments we wish to compare. For example, different substrates for growth might be supplied alternatively through time in one environment, while they are supplied simultaneously in another (Chapter 2). Genetic variance in fitness will arise in the population through mutation. It is expected that some of the mutations will be beneficial, in the sense that they improve fitness under the prevailing conditions of growth, and that they will increase in frequency through selection, thus leading to adaptation. Fitness in these experiments is defined as the rate of change in frequency of a genotype relative to its ancestor and is commonly measured in head-to-head competitions between a genotype of interest and its ancestor. Most often the genotypes are competed in the environments that were used in the selection experiment. The amount of diversity in the selected populations can then be scored by using the genotype-by-environment interaction.

In this thesis I use microbial experimental evolution to explore two extensions of the model of the maintenance of diversity in heterogeneous environments outlined above. Environmental variation is central to our interpretation of diversity but remains poorly understood.
In Chapter 1 therefore, I develop a framework to evaluate the importance of two characteristics of the environment fundamental for diversity: the relative productivity of different patches comprised in a heterogeneous environment, and the degree to which conditions of growth differ among patches, or environmental contrast. By productivity I mean the contribution of a patch to the overall number of individuals produced in the environment. The relative productivity of a patch is important because it influences the relative rate of migration of individuals from one patch to another: productive patches produce more migrants relative to unproductive patches. This process has important consequences for the maintenance of diversity because a large number of individuals migrating from the productive to the unproductive patch will prevent adaptation to the unproductive patch (Maynard Smith

and Hoekstra 1980). In Figure 1*C* for example, one genotype (empty dots) may be eliminated by selection even though it grows faster on one of the substrates (no. 1). This happens because the other genotype (filled dots) has a superior average fitness across both patches. Therefore a higher fitness in a subset of the environment is necessary but not sufficient to maintain diversity. However differences in productivity need not preclude the maintenance of diversity as deviation between genotypes may be small (Figure 1 *D*).

The second aspect of environmental variation studied in Chapter 1, the environmental contrast, reflects the degree to which conditions of growth differ among patches. Conditions of growth can be defined in terms of properties intrinsic to the environment, such as shade and light gaps, or in terms of the response of the organisms. The response can be either ecological, for example when patches differ in the average growth rate they sustain, or it can be evolutionary. I have used an evolutionary measure because it is easily quantifiable and is the most appropriate when new genotypes are introduced through mutation. The environmental contrast is thus measured from the direct and correlated responses to selection. The direct response corresponds to the fitness of selected genotypes in the selection environment, while the correlated response is the fitness measure in a second environment that was not experienced during selection. The environmental contrast will be large when the correlated response is negative and small when it is positive. In the experiment, environmental contrast and relative productivity were measured for a number of pairs of substrates and diversity was allowed to arise in a clonal population selected in all pair-wise mixtures of the substrates.

In Chapter 2, I study the effect of the grain of the environment on the evolution of diversity. Environments are said to be fine-grained when individuals have access to alternative resources within their lifetime. Alternatively, environments are coarse-grained when access is restricted to a subset of all possible resources within individual lifetime, but unrestricted when lineages instead of individuals are considered. Spatially coarse-grained environments offer a refuge to specialists and are thus expected to maintain diversity. The opportunity for diversification in spatially fine-grained environment is much more restricted (Maynard Smith 1998). Among other things, the outcome of the experiment showed a consistent bias towards one of the substrate present in the selection environments, a result that was not predicted. This result suggested a potential bias in the supply rate of beneficial mutation for this substrate, which was confirmed by supplementary experiments described in Chapter 2. Overall, these results suggest that the outcome of selection in heterogeneous environments may be impacted by the underlying supply of mutations.

Literature cited

Ackermann, M., and M. Doebeli. 2004. Evolution of niche width and adaptive diversification. Evolution 58:2599-2612.

Bell, G. 1997a. Selection: The Mechanism of Evolution. Chapman and Hall, New York.

Bell, G., Lechowicz, M.J. , Appenzeller, A., Chandler, M., DeBlois, E., Jackson, L., Mackenzie, B., Preziosi, R., Schallenberg, M., and N. Tinker. 1993. The spatial structure of the environment. Oecologia 96:114-121.

Bell, G., Lechowicz, M. J., and M. J. Waterway. 2000. Environmental heterogeneity and species diversity of forest sedges. Journal of Ecology 88:67-87.

Bolnick, D.I., Svanbäck, R., Fordyce, J.A., Yang, L.H., Davis, J.M., Hulsey, C.D., and M.L. Forister. 2003. The ecology of individuals: Incidence and implications of individual specialization. American Naturalist 161:1-28.

Culotta, E., and E. Pennisi. 2005. Breakthrough of the year: Evolution in action. Science 310:1878-1879.

Hardin, G. 1960. The competitive exclusion principle. Science 131:1292-1297.

Hutchinson, G.E. 1961. The paradox of the plankton. American Naturalist 95: 137-145.

Kassen, R. 2002. The evolution of specialists, generalists, and the maintenance of diversity. Journal of Evolutionary Biology 15:173-190.

Maynard Smith, J. 1998. Evolutionary genetics. Oxford University Press, Oxford.

Maynard Smith, J., & R. Hoekstra. (1980). Polymorphism in a varied environment: how robust are the models? *Genet. Res.*, 35, 45-57.

Ricklefs, R.E. Ecology. Fourth Edition. Freeman and Co., New York.

Schluter, D., and R.E. Ricklefs (Ed.). 1993. Species diversity in ecological communities: historical and geographical perspectives. University of Chicago Press, Chicago.

Silvertown, J. 2005. Demons in Eden: the paradox of plant diversity. University of Chicago Press, Chicago.

Tansley, A.G. 1917. On competition between *Galium saxatile* L. (*G. hercynicum* Weig) and *Galium sylvestrum* Poll. (*G. asperum* Schreb.) on different types of soil. Journal of Ecology 5: 173-179.

Wright, S.J., Muller-Landau, H.C., Condit, R., and S.P. Hubbell. 2003. Gap-dependent recruitment, realized vital rates, and size distributions of tropical trees. Ecology 84:3174-3185.

Chapter 1

On the experimental evolution of specialization and diversity in heterogeneous environments

Second submission to *Ecology Letters*

Abstract

Environmental variance can be decomposed into two components: the contrast among patches in the optimal phenotype and the variance in productivity among patches. Both components can in principle impact the outcome of selection in heterogeneous environments. In general genetic diversity within a population should increase as the contrast among patches increases. As the variance in productivity among patches increases, on the other hand, the niche breadth of any given genotype should decrease. We tested this idea by selecting the bacterium *Pseudomonas fluorescens* over several hundred generations in pair-wise mixtures of four carbon substrates. We then assessed the response to selection in these mixtures separately for each substrate they comprised. The results provide general support for this interpretation of the environmental variance and suggest, moreover, that competitive interactions among genotypes help drive diversification in heterogeneous environments.

INTRODUCTION

The outcome of selection in large populations occupying a spatially variable environment is governed by the balance between the strength of divergent selection and the extent of migration among patches or sites. Diversity is most readily maintained when the different patches comprising a heterogeneous environment are strongly contrasted, such that no single type is fittest under all growth conditions (Bell 1990, 1992; Kassen & Bell 2000; Day 2000), and there is little migration among patches (Maynard Smith & Hoekstra 1980; Hedrick 1986; Habets *et al.* 2006). If neither of these conditions holds, diversity will be lost due to the evolution of either a single broadly-adapted generalist or a specialist adapted to the most productive patch. In general then, diversity will tend to be preserved more readily as the environmental variance in fitness across sites increases.

This view of the connection between diversity and environmental variation can be made more explicit by distinguishing the factors contributing to environmental variance in fitness. Imagine a heterogeneous environment composed of two patches where individuals encounter both patches within their lifetime (the environment is fine-grained *sensu* Levins 1968). The environmental variance in fitness between the patches can be decomposed into two components. The first is the variance in productivity, the number of individuals contributed to the total population by each patch. Variation in the relative contribution of individuals to the total population generates differences in the rate of migration among patches, thus creating a source-sink dynamic between productive and unproductive patches (Pulliam 1988; Holt & Gaines 1992). Adaptation to an unproductive patch is therefore likely to be frustrated by the constant immigration of competitors (and so gene flow if the system is sexual) from the more productive patch (Levins 1968; Maynard Smith & Hoekstra 1980; Via & Lande 1985;

Kawecki & Stearns 1993; Kawecki 1995; Holt 1985, 1996; Whitlock 1996; Holt & Gomulkiewicz 1997; Kawecki & Ebert 2004). The second concerns the contrast in environmental quality between the two patches. If both patches contain the same resources, then genes that are beneficial in one will also be beneficial in the other, and divergence between isolated patches would result only from the stochastic accumulation of neutral mutations (Johnson *et al.* 1995; Travisano *et al.* 1995; Souza *et al.* 1997). As patches become more highly contrasted, perhaps because the resources they contain become more different in terms of the optimal phenotype required to exploit them, then genes that are beneficial in one patch are unlikely to be beneficial to the same extent, and may even be deleterious, in the other patch (Via & Lande 1985; Bell 1990; Kassen & Bell 2000; Kassen 2002; Kawecki & Ebert 2004). Highly contrasted environments should thus tend to maintain diversity because divergent selection will be stronger and the fitness trade-offs that evolve as a result are likely to be underlain by antagonistic pleiotropy.

The outcome of selection in a heterogeneous environment will thus depend on the relative importance of both components to the environmental variance in fitness expressed in the population. This argument can be represented graphically (Fig. 1). Selection in patches of similar quality should lead to the evolution of a single generalist type when the relative production among patches is roughly equal (*a*) and a single specialist type adapted to the most productive patch when productivity is very unequal (*b*). As patches become more highly contrasted, selection becomes more divergent, which will lead to the emergence and maintenance of diversity (large ovals showing a negative correlation on Fig. 1) so long as the relative production among patches is roughly equal (*c*), but can lead to the emergence of a single specialist adapted to the most productive patch when productivity is very imbalanced (*d*).

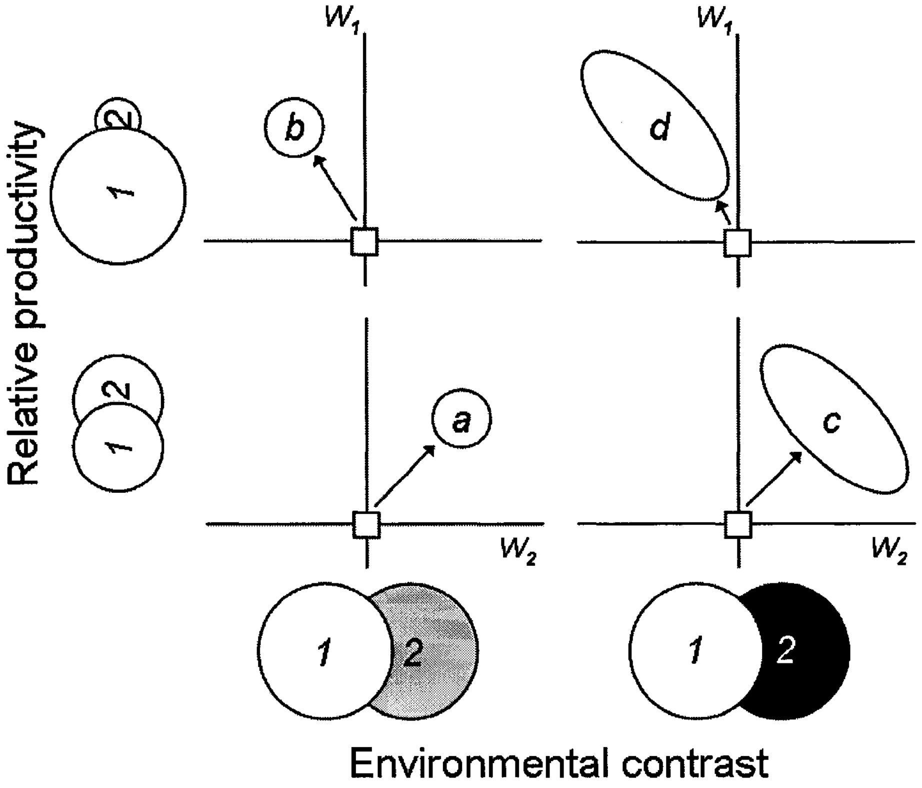

Figure 1 The effect of relative productivity and environmental contrast on the outcome of selection in heterogeneous environments. The response of an ancestral population (square) to selection in a mixture of two resources is given by the location of the circle representing the fitness of different evolved genotypes assayed on resource *1* (W_1) and resource *2* (W_2). The extent of adaptation is determined primarily by the most productive resource when relative productivities are widely divergent (*b*, *d* compared to *a*, *c*, where relative productivity is equal), while the environmental contrast determines the amount of diversity in the population (*c*, *d* maintaining more than *a*, *b*).

Here we evaluate this interpretation of environmental variance for the outcome of selection by following the fate of genetic variation in fitness introduced by mutation in a microbial model system. Our starting material was a single wild type clone of the common soil bacterium *Pseudomonas fluorescens* evolving in fine-grained environments composed of the factorial combinations of four carbon substrates (six pair-wise mixtures) and the appropriate single-substrate controls. This system permits to test the two-patch model outlined above because there is variation in the relative productivity and the environmental contrast between substrate pairs. We asked, first, if the direct response to selection in mixtures occurs primarily in the direction of the most productive substrate and, second, whether genetic variance in fitness is more readily maintained when patches are more highly contrasted. For each pair of carbon substrate, environmental contrast was measured from the direct and correlated responses of populations to selection in single-substrate environments, and relative productivity was measured from the difference in maximal growth rate for the ancestral strain. The relative productivity and the degree of contrast among substrates are therefore measured directly through organismal response. The physiological mechanisms underlying carbon substrate use in *P. fluorescens* are in general not well-understood, precluding the use of more mechanistic interpretations of environmental contrast (Travisano 1997; Ostrowsky *et al.* 2005) except in exceptional circumstances.

Taken together our results provide the first experimental evidence that productive substrates dominate evolution in heterogeneous environments (Wiens & Graham 2005) and that the quantity of diversity arising from new mutations is a function of the environmental contrast. These observations thus provide a framework for interpreting in terms of two ecological processes (productivity and environmental contrast) the results of previous experiments that have considered diversification in heterogeneous environments (for example, Barrett *et al.* 2005). Furthermore, we find evidence suggesting that competition among genotypes drives diversification in a manner reminiscent of character displacement (Maclean *et al.* 2005).

MATERIAL AND METHODS

Study system

Duplicate lines for each selection treatment were founded from a single genotype of the soil bacterium *Pseudomonas fluorescens*, either the wild type strain SBW25 (Rainey and Bailey 1996) or a strain constructed by Dr Xue-Xian Zhang that is isogenic to the wild type, save for the insertion of the *lacZ* gene. Colonies with *lacZ* are blue on agar plates supplemented with 40 mg/L of 5-bromo-4-chloro-3-indolyl-beta-D-galactopyranoside (X-Gal), and can be easily discriminated from pale yellow wild type colonies. Checks for marker neutrality were performed alongside the assays of the selected lines (for five replicates in each of the four substrates the lowest *P* value for a marker effect was 0.17). The ancestral clone of both strains were frozen in a 16 % (v:v) glycerol solution at - 80°C.

Populations were cultured in M9 minimal salts (NH_4Cl 1 g/L, KH_2PO_4 3 g/L, NaCl 0.5 g/L, Na_2HPO_4 6.8 g/L; supplemented with $CaCl_2$ 15 mg/L, $MgSO_4$ 0.5 g/L). Carbon sources were provided at a final concentration of 1.70×10^{-3} M for the single-substrate environments and the low concentration mixtures (two substrates at 8.48×10^{-4} M per substrate), and 3.39×10^{-2}

M for the high substrate concentration mixtures (two substrates at 1.70 x 10^{-3} M per substrate). The sugars used in our experiment had similar molecular weights therefore the total available substrate for each compound was roughly equal. Cultures were grown in an orbital shaker (150 rpm) at 28 °C, in 24 wells plates (Greiner Bio-One, Cellstar®) with 2 ml of media per well.

Selection experiment and fitness assay

The experiment consisted of two replicates of each of 16 selection regimes. The core of the experiment is a factorial combination of four carbon substrates (six mixtures) at two concentration levels, low and high, and each of the four substrates alone. Low concentration mixtures contained the same total amount of substrate as the single-substrate environments whereas the high concentration mixtures contained twice as much substrate as the single-substrate environments. Thus the opportunity for specialization on each substrate in the high concentration mixtures is the same as it is in single-substrate environments. This treatment was included because there appears to be no clear justification for focusing on mixtures that contain the same total carbon as the single-substrate environments versus those that contain twice the amount of carbon. Moreover this design allows us to test directly for an effect of concentration on the degree of specialization, independent of environmental contrast. Thus, the experiment comprised a total of (6 mixtures x 2 productivity levels + 4 single-substrate lines) x 2 replicates = 32 selection lines. Populations were selected under a serial transfer regime of 12 hours for a total of 112 transfers (~ 600 generations). To account in part for differences in total population size, and so mutation supply rates, across selection regimes we bottlenecked all selection lines to approximately 3, 000, 000 cells by manipulating the volume of culture transferred after first measuring the optical density of cultures at 660 nm using a spectrophotometer (EL$_X$-800, Bio-Tek Instruments Inc., Winooski (VT), USA) and converting this to cell density using a standard curve. Note that mutation supply rates remain to some extent unequal because treatments differed slightly in final population size (at the most by a factor of four).

We estimated the average fitness and the variance in fitness for all populations at the end of the experiment by isolating eight colonies from each selection line and competing them against the ancestral strain with the opposing genetic marker. Colonies isolated from mixtures were assayed separately on both of the component substrates at the same concentration in which they were selected. Colonies from single-substrate lines were assayed on all four substrates. Competitions were performed by first culturing the selected isolate and the appropriate ancestral genotype separately for two growth cycles and then mixing the two strains in a 1:1 ratio (by cell number) to initiate the competition. Competitions were run for three growth cycles (36 hours). Initial and final frequencies of the two strains were estimated by plating on X-gal agar. The environment-specific measure of performance of evolved genotypes was the selection coefficient, s (doubling^{-1}), and was calculated as

$$s = \frac{\ln(f_E)_{final} - \ln(f_E)_{initial}}{doublings} \qquad (1)$$

where f_E is the number of colonies of the evolved genotype relative to the ancestral strain on a plate, before (initial) and after (final) competition, and the denominator is the number of doublings of the ancestral strain in the competition environment in three growth cycles (36 hours).

Analysis

Four variables were analyzed. First, the relative productivity of the two substrates in a mixture was calculated as the difference in maximal growth rate of the ancestor grown on both substrates separately (see single-substrate environments in Tables I and 2). Growth rates were estimated from the maximal slope of the curve relating optical density (at 660 nm) to time in replicate wells read every 3 hours. The maximal growth rate rather than the cell density at stationary phase is the appropriate measure of performance when substrates are replenished before their exhaustion, as in our selection experiments (*data not shown*). The relative productivity of a pair of substrates differed with the total substrate concentration therefore we treat both concentrations separately in our analysis.

Second, we measured the environmental contrast between a pair of substrates using the direct and correlated responses to selection of lines selected in each substrate alone using the quantity of genotype-by-environment interaction variance in fitness (G × E) among genotypes isolated from each selection line (following Robertson 1959):

$$\sigma^2{}_{GE} \quad = \quad (\sigma_{G1} - \sigma_{G2})^2/2 \quad + \quad \sigma_{G1}\sigma_{G2}(1-\rho_{G1G2}) \qquad (2)$$

where σ_{G1} and σ_{G2} are the environmental standard-deviation of the fitness of a set of genotypes in environments 1 and 2, and ρ_{G1G2} is the correlation coefficient of fitness of these genotypes across these environments (see Bell 1990; Barrett *et al.* 2005; Kassen & Bell 2000). This quantity thus measures the effectiveness of selection alone, in the absence of dispersal, in generating specialization. Environmental contrast will thus be large when selection has been effective at generating strong trade-offs in fitness across a pair of environments. For each substrate pair there are four independent selection lines (two replicates in two single-substrate environments), giving four estimates of G × E. We used the average of these four estimates as our measure of environmental contrast.

Our third variable was the quantity of genetic diversity within each line selected in a mixture of substrates which we estimated by calculating G × E using equation (2) for the collection of eight genotypes from a single mixture line assayed across a pair of substrates. This measure of diversity represents the degree of specialization effected by selection on the two carbon substrates in a mixture and is directly comparable to the measure of environmental contrast outlined above. The average G × E in each of the six mixture treatments was calculated from both replicates at high and low substrate concentrations.

Fourth, we measured the extent to which a line selected in a mixture of substrates adapted to one or the other substrate by calculating the Euclidean distance (d) between the response to selection on each substrate of the mixture lines and the direct response to selection of the lines selected on the comparable single substrate (see Fig. 2). This procedure was necessary because selection in single-substrate environments is not always accompanied by a cost to adaptation.

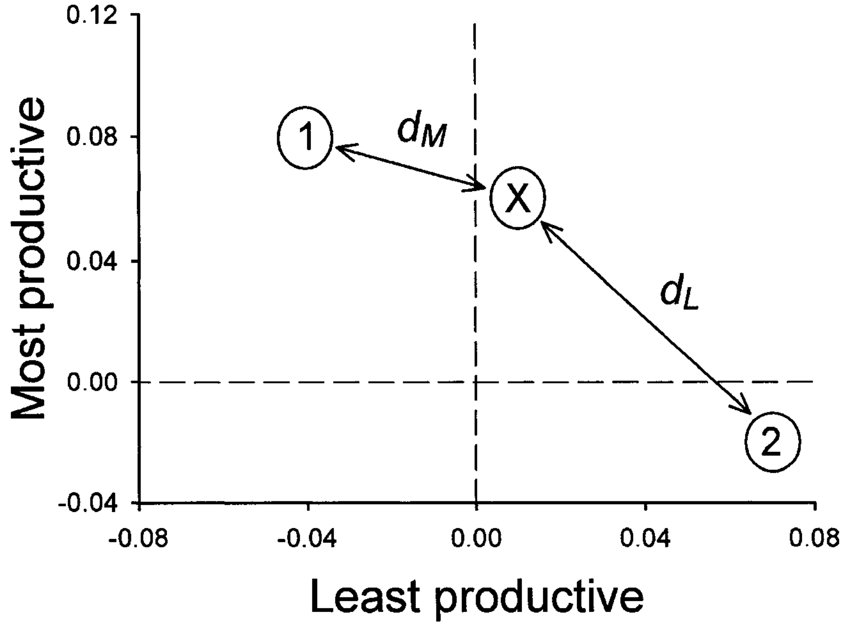

Figure 2 Measuring the response to selection on individual substrates in mixtures. Circles *1* and *2* are the mean fitness of genotypes isolated from populations selected, respectively, in the most productive and least productive substrates, while population *X* was selected in a mixture of both. The arrows represent the Euclidean distance (*d*) between fitness of *X* on both substrates and the fitness of the respective single-substrate selection lines. The populations selected in single-substrate environments constitute 'landmarks' that represents the response to selection to that substrate alone. The relative importance of these substrates for selection in mixtures is thus indicated by the proximity of the lines selected in mixtures to either 'landmark'. Our hypothesis predicts that d_M decreases as the discrepancy in the productivity of two substrates increases.

Re-using substrates in different pairs in the design of the selection experiment and assaying the same selection lines in multiple environments precluded the use of statistics sensitive to the independence of samples. Thus, all *P* values in this paper were generated by performing 10 000 randomizations with a program in Microsoft Visual Basic 6.0 (Microsoft Corp.) that shuffles data by resampling without replacement.

RESULTS

Patterns of growth in the ancestor on single and mixed carbon sources

We assayed the growth of the ancestral clone over the course of a single growth cycle in each of the experimental environments in order to assess the relative productivity of each substrate and to gain insights about the pattern of substrate use in mixtures. Maximal growth rates were faster in mixtures than on their respective component substrates in three out of six cases, independently of the productivity level (Tables 1 and 2). We take this result as support for the idea that both substrates are used simultaneously in these mixtures. In the remaining three mixtures, two patterns were observed. In two cases growth rates on mixtures were intermediate between the two component substrates and in one case the growth rates in the mixture was inferior to both single substrates (filled cells in tables 1 and 2). These results suggest that the metabolism of carbon compounds in these mixtures involves some form of interference, although the precise mechanism by which this occurs is not clear. Both substrates may be used simultaneously, or they may be used sequentially. Note however that in all mixtures save one (mannitol-mannose at both concentrations) the cell density in mixtures at the end of a growth cycle was higher than in the comparable single-substrate environments suggesting that both substrates are used in the course of a growth cycle (*data not shown*).

Table 1 Maximal growth rate on single-substrates (long diagonal) and mixtures at low resource concentration (mean of three replicates ± 1 S.D., in O.D. units per hour). For mixtures, empty cell: growth in mixture is faster than on both single-substrates; dark grey cell: growth in mixture is similar to the best single-substrate; light grey: growth in mixture is lower than on the best single-substrate.

	Glucose	Mannose	Mannitol	Sorbitol
Glucose	0.012 ± 0.002			
Mannose	0.017 ± 0.006	0.007 ± 0.001		
Mannitol	0.038 ± 0.006	0.018 ± 0.010	0.018 ± 0.001	
Sorbitol	0.037 ± 0.004	0.012 ± 0.001	0.015 ± 0.001	0.017 ± 0.002

Table 2 Maximal growth rate on single-substrates (long diagonal) and mixtures at high resource concentration (mean of three replicates ± 1 S.D., in O.D. units per hour). See Table 1 for the interpretation of cells shading.

	Glucose	Mannose	Mannitol	Sorbitol
Glucose	0.019 ± 0.002			
Mannose	0.023 ± 0.006	0.016 ± 0.001		
Mannitol	0.045 ± 0.008	0.012 ± 0.002	0.027 ± 0.001	
Sorbitol	0.042 ± 0.009	0.028 ± 0.001	0.028 ± 0.006	0.031 ± 0.001

Response to selection

The response to selection in all pair-wise combinations of substrates is shown in figure 3. In all but one pair (specifically, figure 3*f*), response to selection in single-substrate environments led to the evolution of a negative genetic correlation in fitness across environments, indicating the evolution of specialists on their respective substrates. This trade-off was often underlain by a cost of adaptation in one of the substrates but not the other. The one exception involved the mannitol-sorbitol substrate pair (Fig. 3*f*) where the correlated responses to selection on both substrates were positive. In most cases, the response to selection at different total concentrations of carbon in mixtures gave comparable results. There were two notable exceptions: selection at low concentrations of both mannitol and sorbitol in combination with mannose led to a positive response on both pairs of substrates whereas at high concentrations, adaptation in the mixture occurred primarily to mannitol and sorbitol, respectively (Fig. 3*d*, *e*).

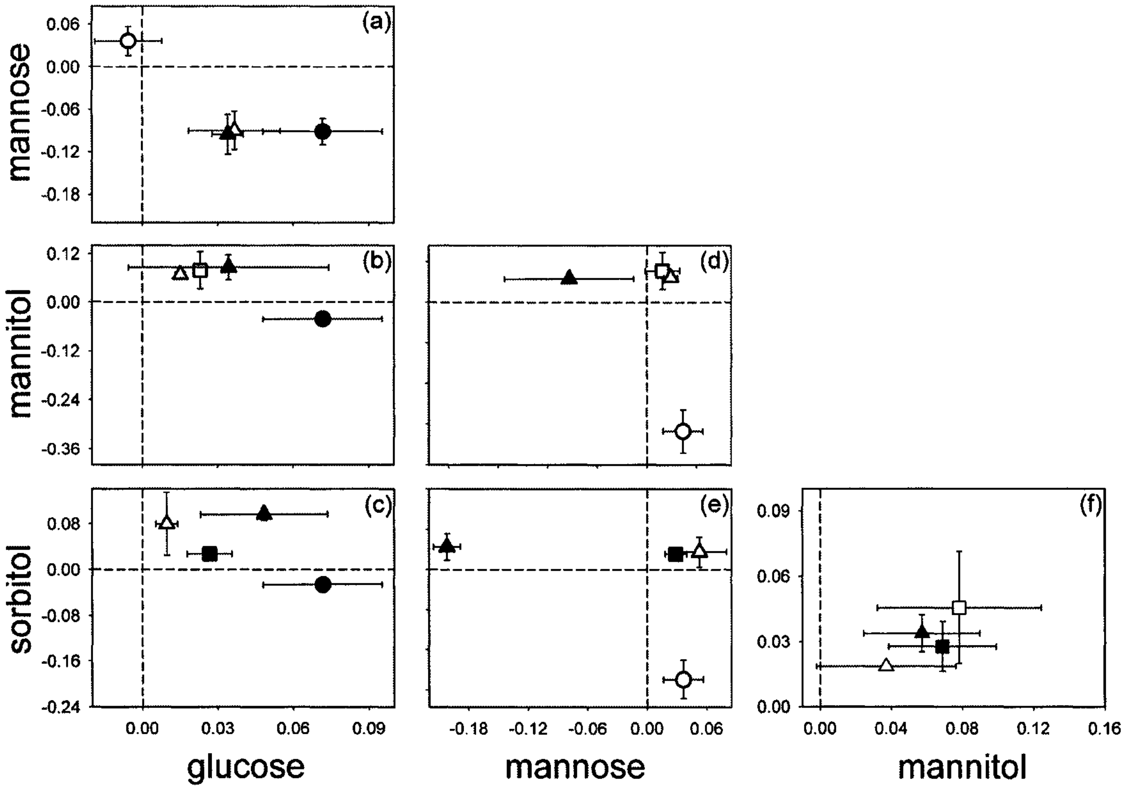

Figure 3 Matrix of the results for the factorial experiment. Each axis represents the selection coefficients (per doubling) for each pair of substrates. Symbols show populations selected in glucose (filled dots), mannose (empty dots), sorbitol (filled squares), and mannitol (empty squares), and in a low (empty triangles) or high (filled triangles) concentration mixture of both substrates on a panel. Bars show 1 S.E.M.

Productivity and environmental contrast

The response to selection of lines selected in mixtures tended towards the most productive substrate in the mixture, as measured by the Euclidean distance (Fig. 2) between the response to selection of single-substrate lines and mixture lines assayed on the component substrates (one-tailed paired *t*-test comparing d_M against d_L, low concentration mixtures: $t_5 = 2.7$, $P = 0.020$; high concentration mixtures: $t_5 = 2.8$, $P = 0.018$; the six mixtures are not independent as they form the factorial combination of four substrates, however the *t* values remain below $P = 0.05$ unless $d.f. \leq 2$, which is the minimal number of independent comparisons in the preceding test). Note that mixtures at both substrate concentrations gave similar results (paired-*t* test comparing d_M at low *vs.* high substrate concentration $t_5 = 1.4$, $P = 0.23$). Furthermore, we found a quantitative relationship between the difference in productivity and the extent of specialization in mixtures: as the productivity of the two substrates diverges, the Euclidean distance between the phenotype evolved in the mixture and the most productive substrate decreases (Fig. 4*a*). These results provide support for the idea that the extent to which a lineage adapts to the component patches of a heterogeneous environment depends on their relative productivity, adaptation occurring most readily to the most productive patch.

The extent to which populations selected in mixtures adapted to the most productive substrate also depended on the degree of environmental contrast between the substrates. The Euclidean distance between lines selected in mixtures and those selected on the most productive substrate alone shows a positive relationship with the environmental contrast (Fig. 4*b*). This result suggests that the importance of relative productivity in determining the response to selection depends on the environmental contrast. Note also that our measures of difference in productivity (Δ productivity) and environmental contrast (G x E from the response to selection in single-substrate environments) were not strongly correlated (slope ± 1 S.E.M. = -0.055 ± 0.033, $n = 6$, $r^2 = 0.42$, $P = 0.17$).

We excluded two selection treatments (high concentration mixtures of mannose-mannitol and mannose-sorbitol) from the two regression analysis involving d_M (Fig. 4*a*, *c*). Our rationale is that these lines were clearly more specialized to the most productive substrate (see Fig. 3*d*, *e*) – and indeed paid a large cost of adaptation on the least productive substrate – but the Euclidean distance from the mixture to the more productive substrate was large (including these data points in the analysis does not change the overall pattern but the relationship becomes formally non-significant; slope ± 1 S.E.M. = - 4.9 ± 4.3, $n = 12$, $r^2 = 0.12$ $P = 0.14$; see results presented in Fig. 4*a* for comparison).

Although there is a slight tendency for a negative relationship between relative productivity and diversity, this relationship was not significant (Fig. 4*c*). This result suggests that the relative productivity of different patches may have little impact on the quantity of diversity maintained in a population. There was a significant positive correlation, however, between genetic diversity in mixtures and environmental contrast (Fig. 4*d*), lending support to the idea that diversity is more likely to be maintained when environments are strongly contrasted (Bell 1990; Kassen & Bell 2000). There was a tendency for those mixtures showing clear evidence of simultaneous resource use to support more genetic diversity than mixtures in which only one substrate may be used (mean G × E at low concentration (± 95% confidence-interval) = 0.0027 ± 0.0026 (simultaneous) *vs* 0.0013 ± 0.0012 (non-simultaneous); at high concentration = 0.0020 ± 0.0015 (simultaneous) *vs* 0.0018 ± 0.0018 (non-simultaneous)), however this effect was not significant (ANOVA, simultaneous/non-simultaneous $F_1 = 0.77$, $P = 0.41$; resource concentration $F_1 = 0.0083$, $P = 0.93$; interaction $F_1 = 0.38$, $P = 0.56$).

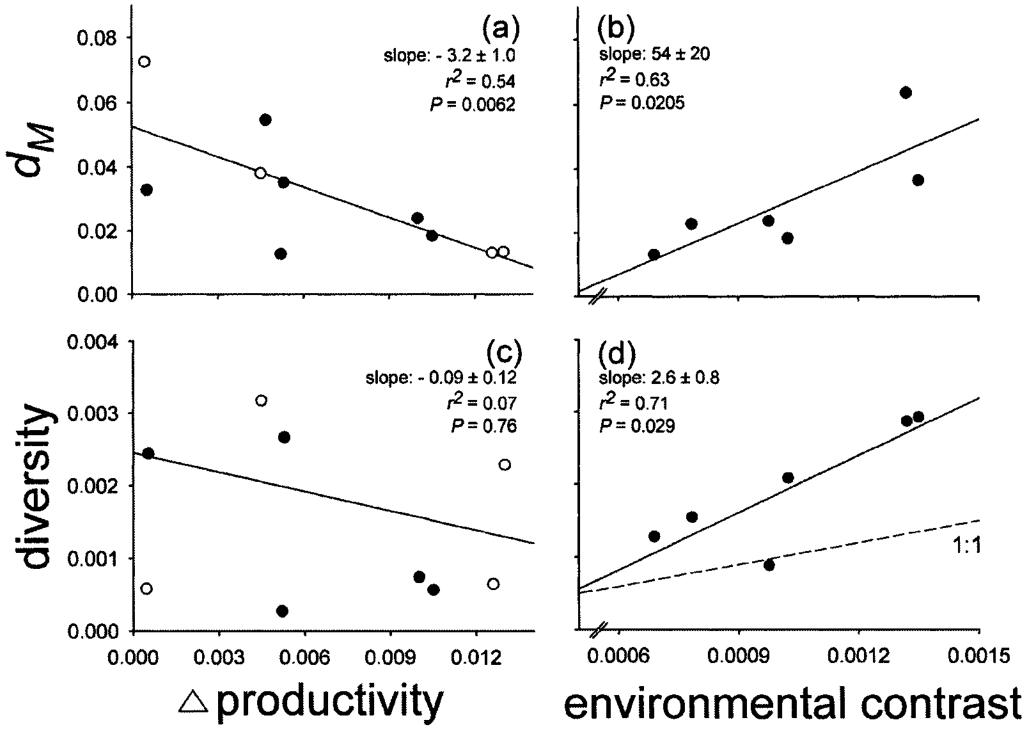

Figure 4 The effect of relative productivity and environmental contrast on d_{M} (the Euclidean distance between the response to selection in mixtures and the most productive single substrate; see Fig. 2) and diversity in mixtures. On panels *a* and *c*, filled dots and empty dots are from low and high concentration mixtures, respectively. Error is ± 1 S.E.M. The *P* values were calculated following a randomization procedure (see methods).

Interestingly, we can take our analysis of figure 4*d* one step further. If the dynamics of selection in mixtures is a simple consequence of adaptation to each component environment then we would expect the quantity of G × E in mixtures (that is, diversity) to be comparable to, or even less than, the G × E generated by selection in the single-substrate environments (our measure of environmental contrast). Alternatively, if competition among genotypes drives diversification, as it does during adaptive radiation (Schluter 2000), then we would expect the G × E expressed in mixtures to exceed, on average, that expressed by the single-substrate lines. These two alternatives can be evaluated by examining the data in figure 4*d* in relation to the 1:1 line. G × E was on average larger in the mixtures than in the single-substrate environments (paired *t*-test, $t_5 = 3.5$, $P = 0.008$), suggesting that competition between genotypes must have been important in driving diversification in these lines.

DISCUSSION

The evolution of specialization and costs of adaptation

In large populations natural selection in a single, uniform environment is expected to lead to the evolution of types that are specialized to the prevailing conditions of growth and who pay a cost of adaptation in terms of their fitness in environments in which they have not been selected (Futuyma & Moreno 1988; Cooper & Lenski 2000). As a consequence of this process, the genetic correlation in fitness across environments should evolve to be negative (Via & Lande 1985; Kassen 2002). Our results broadly support this interpretation of the evolution of specialization and the genetic correlation. We observed the evolution of specialization in all substrates and this often came at a cost of adaptation in alternative environments (Fig. 3).

However this was not always the case. In one pair of substrates, mannitol and sorbitol, the direct and correlated responses to selection on both substrates were positive, leading to a positive genetic correlation in fitness (Fig. 3*f*). It is known that the uptake and utilization of both substrates is under control of the same operon in *P. fluorescens* (Brunker *et al.* 1998), lending support to the idea that the pleiotropic effects of beneficial mutations on one substrate are likely to be positive on other substrates that share similar underlying genes. A similar result has been obtained previously by Travisano and Lenski (1996). Note, however, that there is also evidence for the opposite effect: selection on different substrates that utilise the *lac* operon in *E. coli* has been shown to generate antagonistic pleiotropy (Lunzer *et al.* 2002; Dykhuizen & Dean 2004; Zhong *et al.* 2004). The underlying reason for these different results remains unclear.

Furthermore, costs of adaptation were often asymmetric, in the sense that one specialist paid a cost while the other did not (Fig. 3*b*-*e*). Asymmetric costs of adaptation have often been observed in selection experiments (Bennett *et al.* 1992; Bennett & Lenski 1996; Travisano 1997; Crill *et al.* 2000; Kassen 2002; Greene *et al.* 2005), however their causes are not well understood. The causes of such asymmetry must lie in the biochemical and physiological details underlying the response to selection and the pleiotropic effects of modifications to these systems (Travisano & Lenski 1996; Poon & Chao 2006). Unfortunately we are unable to gain further mechanistic insight into our results because little data exists on the physiological mechanisms responsible for different carbon substrate use in *P. fluorescens*, save for the case of mannitol and sorbitol discussed in the preceding paragraph. Why the pleiotropic effects of selection on different characters would differ so markedly is one of the greatest challenges of experimental evolution (Rose *et al.* 1996, 2005) and has broad implications for our understanding of diversity.

Interestingly, the costs of adaptation that evolved in mixtures sometimes depended on the total carbon concentration available. In two cases (Fig. 3*d*, *e*), lines selected at high concentrations of carbon paid a fitness cost on the less productive substrate that was not paid by lines selected at low carbon concentrations or by the lines selected on the comparable single-substrate. The causes of this effect are not clear but one possible explanation is that the loss of the ability to metabolize the less productive substrate conferred a fitness advantage on the more-productive substrate. A result similar to this has been noted before (see for example Cooper & Lenski 2000; Cooper *et al.* 2001). Moreover, a similar phenomenon seems to have been noted from at least two field studies that have contrasted performance in rich and poor habitats (Stearns & Sage 1980; Dias & Blondel 1996).

Productivity and environmental contrast

We have explored an interpretation of the environmental variance that sees it as being composed of two parts. The first is the relative productivity of different patches of the environment, represented by the numbers of individuals contributed to the total population through growth on any given patch. The second is the environmental contrast, the degree to which patches differ in the qualitative conditions of growth offered. Our results suggest that both components impact the outcome of selection in heterogeneous environments.

The dominant effect of variance in relative productivity is to determine the direction of the average response to selection (Levins 1968; Holt 1985; Kawecki & Ebert 2004). In our experiment, lines selected in mixtures tended to be better-adapted to the most productive substrate of the mixture, and this effect became more pronounced as the disparity in productivity between substrates increased (Fig. 4*a*). This result lends experimental support to the idea of niche conservatism (Holt & Gaines 1992; Holt 1996; Peterson *et al.* 1999), where demographic asymmetries among patches constrain the evolution of niche breadth to be narrower than it would otherwise be based on the qualitative differences among patches. To the best of our knowledge, ours is the first experimental evolutionary evidence for niche conservatism (Wiens & Graham 2005). Furthermore, these results are reminiscent of the zero-one rule in optimal foraging theory, which predicts that organisms will not exploit resources that do not contribute a net benefit to individual growth (Macarthur & Pianka 1966; Stephens & Krebs 1986). In our experiment lines selected in mixtures showed little evidence of adaptation to the less productive substrate, despite this substrate being available (paired *t*-test comparing correlated response on the less productive substrate of lines selected on the most productive substrate with the response to selection of the mixture lines on the less productive substrate: low concentration mixtures: $t_5 = -1.2$, $P = 0.85$; high concentration mixtures: $t_5 = -1.7$, $P = 0.92$). This result suggests that the effect of productivity on adaptation resulted in an effective 'rejection' of the less productive substrate.

The primary effect of increasing environmental contrast, on the other hand, is to support increasing levels of diversity. On average, we found more genetic diversity (measured as genotype-by-environment interaction variance) maintained in populations that had been selected in mixtures when contrasted to those selected in single-substrate environments, a result that has been observed previously (see Bell & Reboud 1997; Kassen 2002; Barrett *et al.* 2005; Barrett & Bell 2006). Moreover, we have shown that the quantity of diversity in mixtures increases with the increasing disparity in qualitative conditions of growth among patches (Fig. 4*d*) through the selection of *de novo* variation arising from mutation. Although comparable results on ecological time scales have been obtained by Bell (1990) and Kassen & Bell (2000), where selection sorts among extant genetic variation, ours appears to be the first experiment to have considered this effect on evolutionary time scales where mutation is the sole source of variation. Taken together, these experiments provide strong evidence that diversity should be more readily supported when environments are highly heterogeneous.

An alternative explanation for the variation in genetic diversity among mixture populations is possible. If the cellular machinery necessary to metabolise a substrate is repressed in the presence of another substrate, a process called catabolite repression (see Collier *et al.* 1996), bacteria will often first use one substrate and then switch to an alternative substrate once the first is exhausted, creating what amounts to a temporally variable environment. The conditions for the maintenance of diversity in temporally varying environments are generally much more restrictive than in spatially varying ones (Hedrick 1986; Felsenstein 1976; Doebeli 2002), suggesting that lower levels of diversity would be supported under catabolite repression. Although there was a tendency in our experiment for diversity to be lower in mixtures where catabolite repression may have been operating, this difference was not statistically significant. Furthermore, it is difficult to see how the catabolite repression hypothesis can account for the quantitative relationship between environmental contrast and diversity we observed (Fig. 4*d*), as we would expect mixtures exhibiting catabolite repression to have levels of diversity that are all similarly low. Thus our results do not seem to be an artefact of differences in the temporal pattern of resource use in mixtures.

Interestingly, we also found that the quantity of diversity in mixtures was more than expected if selection simply sorted among novel variation. These exaggerated differences among genotypes in mixtures lend support to the idea that resource competition drives diversification through character displacement (Brown & Wilson 1956; Schluter 2000; see also Barrett & Bell 2006 for a similar result): individuals from mixtures were more divergent in terms of their fitness on the component substrates than individuals from comparable single-substrate environments.

CONCLUSION

The leading results of our work indicate that predicting the outcome of selection in heterogeneous environments requires we consider both the variance in productivity and the qualitative differences among patches. We have shown that the mean response to selection in environments containing two substrates will be dominated by adaptation to the most productive patch. The quantity of genetic diversity maintained within a population, however, seems to be largely determined by the environmental contrast. This interpretation suggests that, in the short term of a few hundred generations, selection is relatively ineffective at eliminating diversity even in the presence of demographic imbalances among patches of a heterogeneous environment. Diversity in the form of specialists to relatively unproductive patches, then, could be more readily supported than previously thought.

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REFERENCES

Barrett, R.D.H., Maclean, R.C., & G. Bell. (2005). Experimental evolution of *Pseudomonas fluorescens* in simple and complex environments. *Am. Nat.*, 166, 470-480.

Barrett, R.D.H., & G. Bell. (2006). The dynamics of diversification in evolving *Pseudomonas* populations. *Evolution*, 60, 484-490.

Bell, G. (1990). The ecology and genetics of fitness in *Chlamydomonas*. I. Genotype-by-environment interaction among pure strains. *Proc. R. Soc. of Lond. B*, 240, 295-321.

Bell, G. (1992). The ecology and genetics of fitness in *Chlamydomonas*. V. The relationship between genetic correlation and environmental variance. *Evolution*, 46, 561-566.

Bell, G., & X. Reboud. (1997). Experimental evolution in *Chlamydomonas*. V. Genetic variance in strongly contrasted environments. *Heredity*, 78, 498-506.

Bennett, A.F., Lenski, R.E., & J.E. Mittler. (1992). Evolutionary adaptation to temperature. I. Fitness responses of *Escherichia coli* to changes in its thermal environment. *Evolution*, 46, 16-30.

Bennett, A.F., & R.E. Lenski. (1996). Evolutionary adaptation to temperature. V. Adaptive mechanisms and correlated responses in experimental lines of *Escherichia coli*. *Evolution*, 50, 493-503.

Brown, Jr., W.L., & E.O. Wilson. (1956). Character displacement. *Syst. Zool.*, 5, 49-64.

Brunker, P., Altenbuchner, J., & R. Mattes. (1998). Structure and function of the genes involved in mannitol, arabitol and glucitol utilization from *Pseudomonas fluorescens* DSM50106. *Gene*, 206, 117-126.

Collier, D.N., Hager, P.W., & P.V. Phibbs, Jr. (1996). Catabolite repression control in the *Pseudomonads*. *Res. Microbiol.*, 6-7, 551-561.

Cooper, V.S., & R.E. Lenski. (2000). The population genetics of ecological specialization in evolving *Escherichia coli* populations. *Nature*, 407, 736-739.

Cooper, V.S., Schneider, D., Blot, M., & R.E. Lenski. (2001). Mechanisms causing rapid and parallel losses of ribose catabolism in evolving populations of *Escherichia coli* B. *J. Bacteriol.*, 183, 2834-2841.

Crill, W.D., Wichman, H.A., & J.J. Bull. (2000). Evolutionary reversals during viral adaptation to alternating hosts. *Genetics*, 154, 27-37.

Day, T. (2000). Competition and the effect of spatial resource heterogeneity on evolutionary diversification. *Am. Nat.*, 155, 790-803.

Dias, P.C., & J. Blondel. (1996). Local specialization and maladaptation in the Mediterranean blue tit (*Parus caeruleus*). *Oecologia*, 107, 79-86.

Doebeli, M. (2002). A model for the evolutionary dynamics of cross-feeding polymorphisms in microorganisms. *Popul. Ecol.*, 44, 59-70.

Dykhuizen, D.E., & A.M. Dean. (2004). Evolution of specialists in an experimental microcosm. *Genetics*, 167, 2015-2026.

Felstenstein, J. (1976). The theoretical population genetics of variable selection and migration. *Ann. Rev. Genet.*, 10, 253-280.

Futuyma, D.J., & G. Moreno. (1988). The evolution of ecological specialization. *Annu. Rev. of Ecol. Syst.*, 19, 207-233.

Greene, I. P. Wang, E., Deardorff, E.R., Milleron, R., Domingo, E., & S.C. Weaver. (2005). Effect of alternating passage on adaptation of Sindbis virus to vertebrate and invertebrate cells. *J. Virol.*, 79, 14253-14260.

Habets, M.G.J.L., Rozen, D.E., Hoekstra, R.F., & J.A.G.M. de Visser. (2006). The effect of population structure on the adaptive radiation of microbial populations evolving in spatially structured environments. *Ecol. Lett.*, 9, 1041-1048.

Hedrick, P.W. (1986). Genetic polymorphism in heterogeneous environments: a decade later. *Annu. Rev. Ecol. Syst.* 17, 535-566.

Holt. R.D. (2002). Population dynamics in two-patch environments: some anomalous consequences of an optimal habitat distribution. *Theor. Pop. Biol.*, 28, 181-208.

Holt, R.D. (1996). Adaptive evolution in source-sink environments: direct and indirect effects of density-dependence on niche evolution. *Oikos*, 75, 182-192.

Holt, R.D., & M.S. Gaines. (1992). Analysis of adaptation in heterogeneous landscapes: implications for the evolution of fundamental niches. *Evol. Ecol.*, 6, 433-447.

Holt, R.D., & R. Gomulkiewicz. (1997). How does immigration influence local adaptation? A reexamination of a familiar paradigm. *Am. Nat.*, 149, 563-572.

Johnson, P.A., Lenski, R.E., & F.C. Hoppensteadt. (1995). Theoretical analysis of divergence in mean fitness between initially identical populations. *Proc. R. Soc. Lond. B*, 259, 125-130.

Kassen, R., & G. Bell. (2000). The ecology and genetics of fitness in *Chlamydomonas*. X. The relationship between genetic correlation and genetic distance. *Evolution*, 54, 425-432.

Kassen, R. (2002). The evolution of specialists, generalists, and the maintenance of diversity. *J. Evol. Biol.*, 15, 173-190.

Kawecki, T.J. (1995). Demography of source-sink populations and the evolution of ecological niches. *Evol. Ecol.*, 9, 38-44.

Kawecki, T.J., & S.C. Stearns. (1993). The evolution of life histories in spatially heterogeneous environments: optimal reaction norms revisited. *Evol. Ecol.*, 7, 155-174.

Kawecki, T.J., & D. Ebert. (2004). Conceptual issues in local adaptation. *Ecol. Lett.*, 7. 1225-1241.

Levins, R. (1968). Evolution in changing environments. Princeton University Press, Princeton.

Lunzer, M., Natarajan, A., Dykhuizen, D.E., & A.M. Dean. (2002). Enzyme kinetics, substitutable resources and competition: from biochemistry to frequency-dependent selection in *lac*. *Genetics*, 162, 485-499.

MacArthur, R.H., & E.R. Pianka. (1966). On optimal use of a patchy environment. *Am. Nat.*, 100, 603-609.

Maclean, R.C., Dickson, A., & G. Bell. (2005). Resource competition and adaptive radiation in a bacterial microcosm. *Ecol. Lett.*, 8, 38-46.

Maynard Smith, J., & R. Hoekstra. (1980). Polymorphism in a varied environment: how robust are the models? *Genet. Res.*, 35, 45-57.

Ostrowski, E.A., Rozen, D.E., & R.E. Lenski. (2005). Pleiotropic effects of beneficial mutations in *Escherichia coli*. *Evolution*, 59, 2343-2352.

Peterson, A.T., Soberón, J., & V. Sánchez-Cordero. 1999. Conservatism of ecological niches in evolutionary time. *Science*, 285, 1265-1267.

Poon, A.F.Y., & L. Chao. (2006). Functional origins of fitness effect-sizes of compensatory mutations in the DNA bacteriophage ΦX174. *Evolution*, 60, 2032-2043.

Pulliam, H.R. (1988). Source, sinks, and population regulation. *Am. Nat.*, 132, 652-661.

Rainey, P.B., & M.J. Bailey. (1996). Physical and genetic map of the *Pseudomonas fluorescens* SBW25 chromosome. *Mol. Microbiol.*, 19, 521-533.

Robertson, A. (1959). The sampling variance of the genetic correlation coefficient. *Biometrics*, 15, 469-485.

Rose, M.R., Nusbaum, T.J., & A.K. Chippindale. (1996). Laboratory evolution: the experimental wonderland and the Cheshire cat syndrome. In: *Adaptation*. (ed. Rose, M.R. & Finch C.E.). Academic Press, New York, pp. 221-241.

Rose, M.R., Passananti, H.B., Chippindale, A.K., Phelan, J.P., Matos, M., Teotonio, H., & L.D. Mueller. (2005). The effects of evolution are local: evidence from experimental evolution in *Drosophila*. *Integr. Comp. Biol.*, 45, 486-491.

Schluter, D. (2000). Ecological character displacement in adaptive radiation. *Am. Nat.*, 156, S4-S16.

Souza, V., Turner, P.E., & R.E. Lenski. (1997). Long-term experimental evolution in *Escherichia coli.* V. Effects of recombination with immigrant genotypes on the rate of bacterial evolution. *J. Evol. Biol.*, 10, 743-769.

Stearns, S.C., & R.D. Sage. (1980). Maladaptation in a marginal population of the mosquito fish, *Gambusia affinis*. *Evolution*, 34, 65-75.

Stephens, D.W., & J.R. Krebs. (1986). Foraging theory. Princeton University Press, Princeton.

Travisano, M. (1997). Long-term experimental evolution in *Escherichia coli.* VI. Environmental constraints on adaptation and divergence. *Genetics*, 146, 471-479.

Travisano, M., & R.E. Lenski. (1996). Long-term experimental evolution in *Escherichia coli*: IV. Targets of selection and the specificity of adaptation. *Genetics*, 143, 15-26.

Travisano, M., Vasi, F., & R.E. Lenski. (1995). Long-term experimental evolution in *Escherichia coli.* III. Variation among replicate populations in correlated responses to novel environments. *Evolution*, 49, 189-200.

Via, S., & R. Lande. (1985). Genotype-Environment interaction and the evolution of phenotypic plasticity. *Evolution*, 39, 505-522.

Whitlock, M.C. (1996). The Red Queen beats the Jack-Of-All-Trades: the limitations on the evolution of phenotypic plasticity and niche breadth. *Am. Nat.*, 148, S65-S77.

Wiens, J.J., & C.H. Graham. (2005). Niche conservatism: integrating evolution, ecology, and conservation biology. *Annu. Rev. Ecol. Evol. Syst.*, 36, 519-539.

Zhong, S., Khodyrsky, A., Dykhuizen, D.E., & A.M. Dean. (2004). Evolutoinary genomics of ecological specialization. *Proc. Natl. Acad. Sci.*, 101, 11719-11724.

Chapter 2

Selection in heterogeneous environments is constrained by the mutational landscape in a bacterial evolutionary experiment

Submitted to the *American Naturalist*

Abstract

The classical interpretation of the evolution of ecological specialization is that it results from trade-offs in fitness across environments caused ultimately by costs of adaptation. Experimental evidence for the existence of costs of adaptation is often lacking, however, and this has led many authors to question their importance in the evolution of the niche. Here we provide experimental evidence that niche specialization is constrained by the mutational landscape available to selection, rather than costs of adaptation. We selected a clonal population of the soil bacterium *Pseudomonas fluorescens* in different kinds of heterogeneous environment composed of a productive and an unproductive resource. We found that, contrary to expectation, populations of specialists to the least productive resource often evolved. Estimates of the rate of fixation and the availability of beneficial mutations revealed a larger supply of beneficial mutants in the less productive resource when compared to the more productive resource. Our results suggest that the mutational landscape available to selection can constrain adaptive evolution in heterogeneous environments through the supply of emerging beneficial mutations.

Introduction

The evolution of the ecological niche of a genotype or species – the range of conditions under which it can grow and reproduce – has traditionally been viewed as the result of two processes (Futuyma and Moreno 1988). Environmental variation, on the one hand, causes divergent natural selection that favours the evolution of ever-broader degrees of adaptation. Because the environmental variation experienced by a population tends to increase through time and space (Bell et al. 1993), selection will tend to favour the evolution of broadly-adapted generalists. This tendency is thought to be constrained, ultimately, by the deleterious effects of genes substituted by selection (antagonistic pleiotropy) or drift (mutation accumulation) leading to the evolution of more narrowly-adapted types underlain by a cost of adaptation and, under certain conditions, the maintenance of diversity (Levins 1968; Cooper and Lenski 2000; Ackermann and Doebeli 2004).

This view of niche evolution has been questioned on two counts. First, field experiments often fail to demonstrate the sorts of trade-offs in fitness across environments expected to be associated with niche specialization (Futuyma and Moreno 1988; Bell et al. 2000; Zhan et al. 2003). Indeed, this lack of strong evidence for trade-offs has led, in part, to the development of a neutral theory of diversity in ecology (Hubbell 2001; Bell et al. 2006). Second, the results of laboratory experiments following the fate of genetic variation in environments that vary in space and time suggest that costs of adaptation either do not readily evolve or, when they do, are typically small (Kassen 2002) and can be rapidly compensated by second-site mutations (Levin et al. 2000; Moore et al. 2000; Maclean et al. 2004; Poon and Chao 2005). It is interesting that in the most comprehensive experiment on the evolution of specialization, by

MacLean and Bell (2002), the average correlated response to selection was positive (see also Bennett et al. 1992; Jaenike 1990; Velicer 1999; Elena 2002). Moreover, environmental variation seems only to slow the rate at which genetic variation is lost from a population (Bell 1997) or to favour the evolution of broadly adapted generalists who show little of the expected 'jack-of-all-trades' trade-off, where fitness in any given environment is traded-off against breadth of adaptation across many environments.

Taken together, these results suggest that costs of adaptation may be less important in constraining the evolution of the niche than previously thought. This point was recognized by Whitlock (1996) who suggested that narrowly-adapted specialists will tend to evolve faster than more broadly-adapted specialists even in the absence of a negative correlation in fitness across environments simply because of demographic constraints imposed on generalists. Here we provide evidence for another source of constraint on the evolution of niche breadth, namely, the mutational landscape from which selection sorts among novel beneficial mutations.

To address the nature of the constraints on the evolution of the ecological niche, we conducted a selection experiment that examines the fate of emerging genetic variation in heterogeneous environments from an initially isogenic state. This experiment was designed to evaluate the importance of the scale of spatial variation relative to the generation time of individuals on niche evolution, and the quantity of diversity maintained in populations. Environments that vary on time scales shorter than a single generation are said to be fine-grained, while those that vary on scales longer than a single generation are considered coarse-grained (Levins 1968). Spatial variation at different scales is expected to impact the diversity of niche specialists that evolve (Gillespie 1974): coarse-grained spatial variation offers refuges from selection for types that are well-adapted to some conditions but not others, facilitating the evolution of niche specialization (Dempster 1955; Day 2000). Under fine-grained variation, diversity cannot usually be maintained because selection will cause adaptation to the most productive resource (Levins 1968; Strobeck 1975; Bolnick 2001; Jasmin and Kassen, *unpublished manuscript*). When the environment is spatially coarse-grained the range of relative productivities among patches that can support diversity is much wider. Selection can thus support a diverse collection of niche specialists through negative frequency-dependent selection (Levene 1953; Dempster 1955; Maynard Smith & Hoesktra 1980).

We thus expected that spatially coarse-grained environments would harbour more genetic variation in fitness across environments than spatially fine-grained environments composed of the same resources. Our experiment was designed to test this prediction. Our results, however, were surprising: we found little effect of the scale of variation on either niche breadth or diversity. Instead, we consistently observed the evolution of a single niche specialist adapted to one resource but not the other under almost all treatments, a result that prompted us to perform three further experiments investigating the role of constraints imposed by the mutational landscape available to selection on the outcome of niche evolution.

Material and methods

Founding strains and media

The founder was a single colony of the soil bacterium *Pseudomonas fluorescens* SBW25::*lacZ*, which is isogenic to the standard SBW25 strain used in previous experiments (Rainey & Bailey 1996, Kassen and Rainey 2004), save for the insertion of a promoterless *lacZ* allele in a phage region (X.-X. Zhang, *personal communication*). Colonies possessing the *lacZ* insertion turn blue when plated on agar supplemented with 40 mg/L 5-bromo-4-chloro-3-indolyl-beta-D-galactopyranoside (X-Gal) and so can be readily distinguished from the wild type strain, which is pale yellow. Checks for marker neutrality were performed alongside the assays of the selected lines. Contamination was monitored during the experiment by checking periodically for the loss of the *lacZ* marker. No losses were observed. The ancestral clone and periodic samples from the evolving lines were frozen in a 16% (v:v) glycerol solution at - 80°C.

The selection medium consisted of M9 minimal salts (NH_4Cl 1 g/L, KH_2PO_4 3 g/L, NaCl 0.5 g/L, Na_2HPO_4 6.8 g/L,) supplemented with $CaCl_2$ 15 mg/L, $MgSO_4$ 0.5 g/L, and a source of carbon provided at a concentration of 1.70×10^{-3} M. The monosaccharides used were either mannose (306 mg/L), xylose (255 mg/L), or a fine-grained mixture of both (306 mg/L of mannose plus 255 mg/L of xylose). Thus, the fine-grained treatment contained twice as much resource as the other environments. We selected all populations on 24 wells plates (Greiner Bio-One, Cellstar®), with 2 ml of media in each well. Cultures were grown in an orbital shaker (150 rpm) at 28 °C.

Main selection experiment

The experiment consisted of two replicates of each of six selection regimes (see fig. 1) for a total of 12 selection lines. Aliquots were transferred every 12-hours for a total of 101 transfers. Mannose is a much more productive resource than xylose, both in terms of final cell density and population growth rate (fig. 2). In order to equalize the mutation supply rate on both substrates, we ensured the effective population size (N_e) for each treatment was approximately 6 x 10^6 cells by manipulating the volume of media (and so cells) transferred. Here, N_e is the harmonic mean of the minimum and maximum population sizes reached in a growth cycle. We estimated the number of cells at the end of a growth cycle by measuring the optical density at 660 nm using a spectrophotometer (EL_X-800, Bio-Tek Instruments Inc.) and converting this to cell density using a standard curve. Note that this manipulation has no effect on the relative contribution of xylose and mannose to the total population in spatially coarse-grained treatment with unequal patch productivity as it is the total number of cells, rather than the contribution of cells from each patch, that is equalized. Note also that N_e in mannose and xylose were equalized under the temporal variation treatment. The difference in growth rates in the two environments means that lines selected in mannose evolved for approximately 550 generations while those in xylose evolved for about 100 generations.

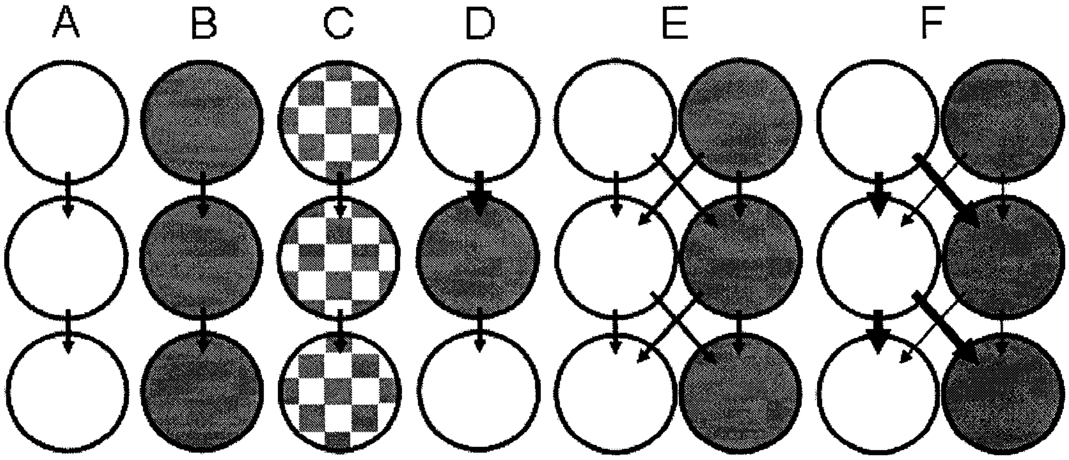

Figure 1: Selection environments. Circles represent microwells containing either xylose (white) or mannose (grey). Arrows represent a transfer; bold arrows represent larger volumes transferred. *A* and *B*, homogeneous environments: aliquots transferred to the same resource, either xylose (*A*) or mannose (*B*); *C*, fine-grained environment: mixture of mannose and xylose; *D*, temporally varying environment: alternation between xylose and mannose; *E*, spatially coarse-grained environment with unequal resource productivity: the contribution of each resource to the total population is a function of its productivity; *F*, spatially coarse-grained environment with equal resource productivity: the fraction of individuals contributed by each carbon source is the same.

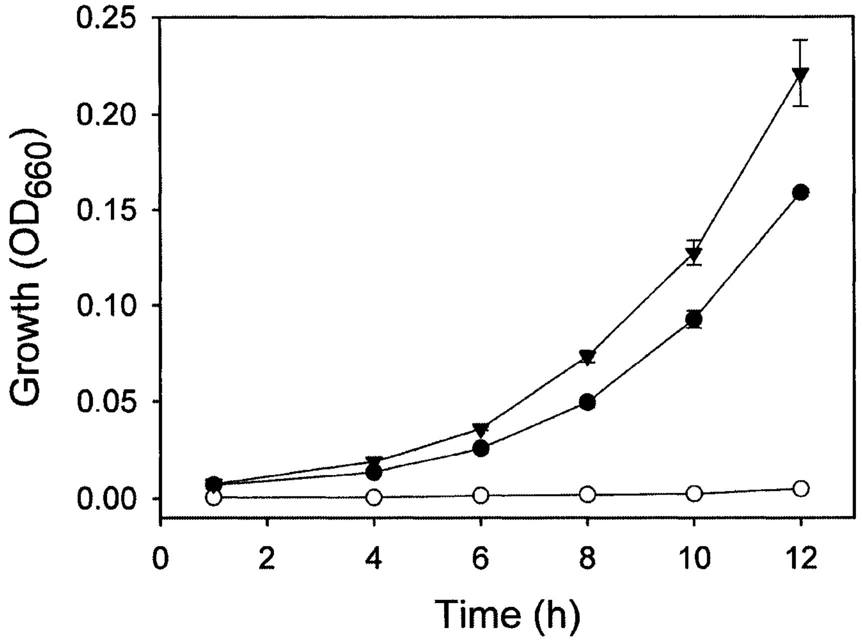

Figure 2: Growth curves of the ancestral genotype in xylose (empty circles), mannose (filled circles), and the mixture (triangles). Mannose is much more productive than xylose and both contribute to growth in the mixture. O.D.$_{660}$ refers to the optical density of the cultures in the wells at a wavelength of 660 nm and is a measure of the number of cells per volume of culture. Data points are average of two replicates ± 1 S.E.M.

Fitness assay

The fitness of the evolved lines was estimated by competing 16 colonies isolated from each selection line against the ancestral genotype lacking the *lacZ* insertion independently in xylose and mannose. All strains including the ancestor were first acclimated from frozen cultures in the environment of competition for two growth cycles and then mixed with the ancestral strain at a 1:1 ratio into the competition environment. Cultures were allowed to compete for four growth cycles, with estimates of the relative frequency of the two types assayed after the first (initial) and fourth (final) growth cycles by plating on agar plates containing M9 minimal salts supplemented with X-Gal. The selection coefficient, *s*, measures the rate at which the frequency of an evolved genotype changes relatively to its ancestor and was calculated as

$$s \quad = \quad \frac{\ln(f_E)_{final} - \ln(f_E)_{initial}}{doublings} \qquad (1)$$

where f_E is the frequency of an evolved genotype relative to the wild type ancestor and doublings refers to the number of doublings by the ancestor in either mannose or xylose in the three growth cycles of the competition experiment. The significance of the response to selection in each environment was assessed with two-tailed *t*-tests. This procedure gives a total of 24 tests against the same ancestral genotype (= 6 treatments x 2 replicate lines x 2 assay environments) so we adjusted significance levels to correct for multiple comparisons using a Bonferoni correction ($\alpha = 0.05/24 = 0.002$). Obtaining separate fitness estimates for the 16 colonies from each line also permits us to estimate the inconsistency component of the genotype-by-environment interaction within each selection line (Robertson 1959; see also Bell 1990; Barrett et al. 2005; Kassen and Bell 2000). The inconsistency measures the amount of crossing in the norms of reactions of the 16 genotypes or, more formally, the lack of correlation across genotypes in a pair of environments. All statistical analyses were performed with JUMP (SAS Institute Inc.).

Rate of fixation experiment

The rate of fixation of new mutations in xylose and mannose was estimated by following the change in marker frequency of populations composed initially of a 1:1 mixture of the two ancestral strains, SBW25 and SBW25::*lacZ*, in each environment for 41 transfers. We established 12 replicate populations in each environment and plated samples from each population every six growth cycles (three days). Transfers were performed in the same manner as for the main selection experiment. The fixation of beneficial mutations can be visualized by monitoring the relative frequency of the two markers in a selected population, as a rare beneficial mutant will arise in one or the other marker background (Hegreness et al. 2006). We estimated the number of transfers after which fixation of one or the other marker occurred as well as the proportion of replicate lines that fix one marker by the end of the experiment. We took fixation to be when one of the two markers comprised at least 95% of the population.

The distribution of fitness among novel mutations

We estimated the distribution of fitness among novel mutants using the library of independently-isolated, single-step mutants of *P. fluorescens* SBW25 reported in Kassen and Bataillon (2006), who provide detailed description of methods for mutant selection. Briefly, mutants arising naturally during population expansion in permissive medium were isolated by antibiotic selection with naladixic acid. The pleiotropic effect of mutations conferring resistance was then measured across a range of environments lacking antibiotic. We chose 46 mutants at random from the 95 fittest mutants in LB medium, the same medium used for antibiotic selection, and estimated the selection coefficient relative to their antibiotic-sensitive ancestor (SBW25::*lacZ*), in both xylose and mannose. Competition experiments were performed as described above. Two replicate estimates of s were obtained for each mutant strain.

Reversion experiment

To assess the source of the cost of adaptation in mannose of lines adapted to xylose, we selected six replicates of each lines previously selected in xylose alone (from the main selection experiment) for 25 transfers in mannose following the same procedure as before. Estimates of fitness were obtained through competition experiments of the entire population against the ancestor in both xylose and mannose. Two replicates of each competition were performed.

Results

Response to selection

Figure 3 shows the response to selection in xylose and mannose alone for the main selection experiment. Populations selected in both homogeneous environments adapted to their environment (fig. 3*A*, *B*; xylose: $t = 13$ and 15, $\mathrm{df} = 15$, $P < 0.0001$; mannose: $t = 7.3$ and 16, df = 15, $P < 0.0001$; H_0: $s = 0$) although the correlated responses to selection were variable. Lines selected on xylose paid a cost of adaptation in mannose ($t = -7.3$ and -20, df = 15, $P < 0.0001$), whereas mannose-selected lines showed little correlated response in xylose ($t = -2.3$, df = 15, $P = 0.039$ and $t = 0.69$, df = 15, $P = 0.70$). Null or negative correlated responses to adaptation are rare in evolutionary experiments with *P. fluorescens* (Barrett et al. 2005; Maclean and Bell 2002), and experimental evolution in general (Kassen 2002; Ostrowski et al. 2005).

The response to selection in the heterogeneous environments was unexpected: in all treatments adaptation occurred to xylose but not mannose (fig. 3*C-F*; all tests: $t > 3.7$, df = 15, $P < 0.002$), save for the unequal patch productivity treatment where the response was positive in both mannose (fig. 3*E*; $t > 3.7$, df = 15, $P < 0.002$) and xylose (although here one population's response was not formally significant: fig. 3*E*, empty dots; $t = 1.7$, df = 15, $P = 0.11$). The overall positive responses to xylose were accompanied by a cost of adaptation to mannose in all cases ($t > - 3.73$, df = 15, $P < 0.002$) but two, which were marginally significant after correcting for multiple comparisons (coarse-grained environment with equal patches productivity: fig. 3*F*; $t = -3.5$, df = 15, $P = 0.0036$ and one replicate line in the temporally variable environment: fig. 3*D*; $t = -2.9$, df = 15, $P = 0.012$).

Finally, the inconsistency component of the genotype-by-environment interaction was largest in the temporal variation treatment (Table I). Recall that the inconsistency is a measure of the amount of crossing in the norms of reactions between selection coefficients in mannose and xylose and estimates the degree to which genotypes specialize on alternative resources. The negative correlation in fitness on xylose and mannose that evolved under this selection regime can easily be seen on fig. 3*D*. The mannose lines also had a surprisingly high inconsistency, although it seems that this is the result of a large variability in both environments rather than a negative correlation across resources. One possible explanation for this result is that one population (filled dots on fig. 3*B*) was sampled in the course of a selective sweep.

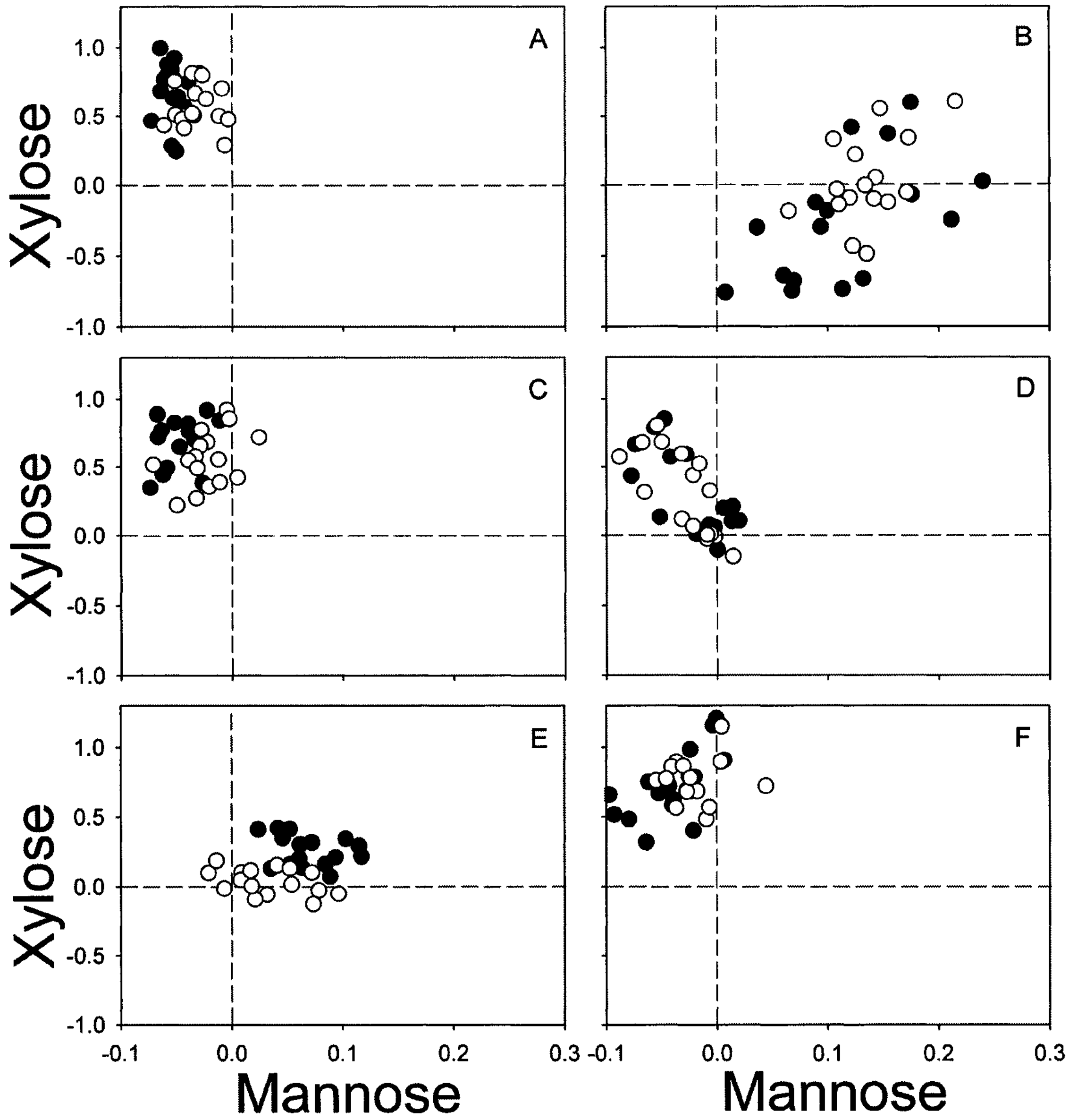

Figure 3: Selection coefficients (per doubling) of genotypes from the main selection experiment. The two replicate selection lines are represented by the filled and open dots, and there were 16 colonies tested from each line. Panels correspond to figure 1: *A*, xylose homogeneous environment; *B*, mannose homogeneous; *C*, spatially fine-grained; *D*, temporal variation; *E*, spatially coarse-grained environment with unequal patches productivity; and *F*, spatially coarse-grained environment with equal patches productivity.

Table 1: The inconsistency component of the genotype-by-environment interaction expressed in mannose and xylose by 16 genotypes isolated in two replicate populations for each treatment of the main selection experiment. Groupings are from a Tukey-Kramer test with $\alpha = 0.05$.

Treatment	group	mean ± 1 S.E.M
Spatially fine-grained	b	0.0025 ± 0.0003
Spatially coarse-grained, equal productivity	b	0.0033 ± 0.0004
Spatially coarse-grained, unequal productivity	b	0.0043 ± 0.0002
Mannose	ab	0.0088 ± 0.0033
Xylose	b	0.0025 ± 0.0003
Temporal variation	a	0.0159 ± 0.0006

Rate of fixation

Figure 4 depicts the change in marker frequency over 41 transfers for lines selected in xylose and mannose. By the end of the experiment, 11/12 lines had fixed one or the other marker in xylose, while only 1/12 lines had fixed a marker in mannose, a difference that is statistically significant ($\chi^2 = 13.5$, df = 1, $P = 0.0002$). This result suggests that the rate of fixation of new beneficial mutations in xylose is faster than in mannose. Among the 11 replicate lines that fixed a marker in xylose at the end of the experiment, six fixed the SBW25 strain and five fixed the SBW25::*lacZ* strain, as expected if the marker was neutral with respect to fitness. It is also possible to test for changes in the marker frequency by regressing, for each replicate line, the marker frequency against the transfer number; this analysis indicated that the average slope in mannose was significantly greater than zero (one-sided *t* test, $t = 6.1$, df = 11, $P < 0.0001$), suggesting that beneficial mutations were also appearing in mannose. Nevertheless even if we assume, very conservatively, that all mannose lines that had not fixed a beneficial mutation by transfer 41 would have done so in the next transfer, the average time to fixation is still significantly shorter in xylose than in mannose (two-sided Wilcoxon signed-rank test, $Z = -3.476$, $N = 24$, $P = 0.0005$).

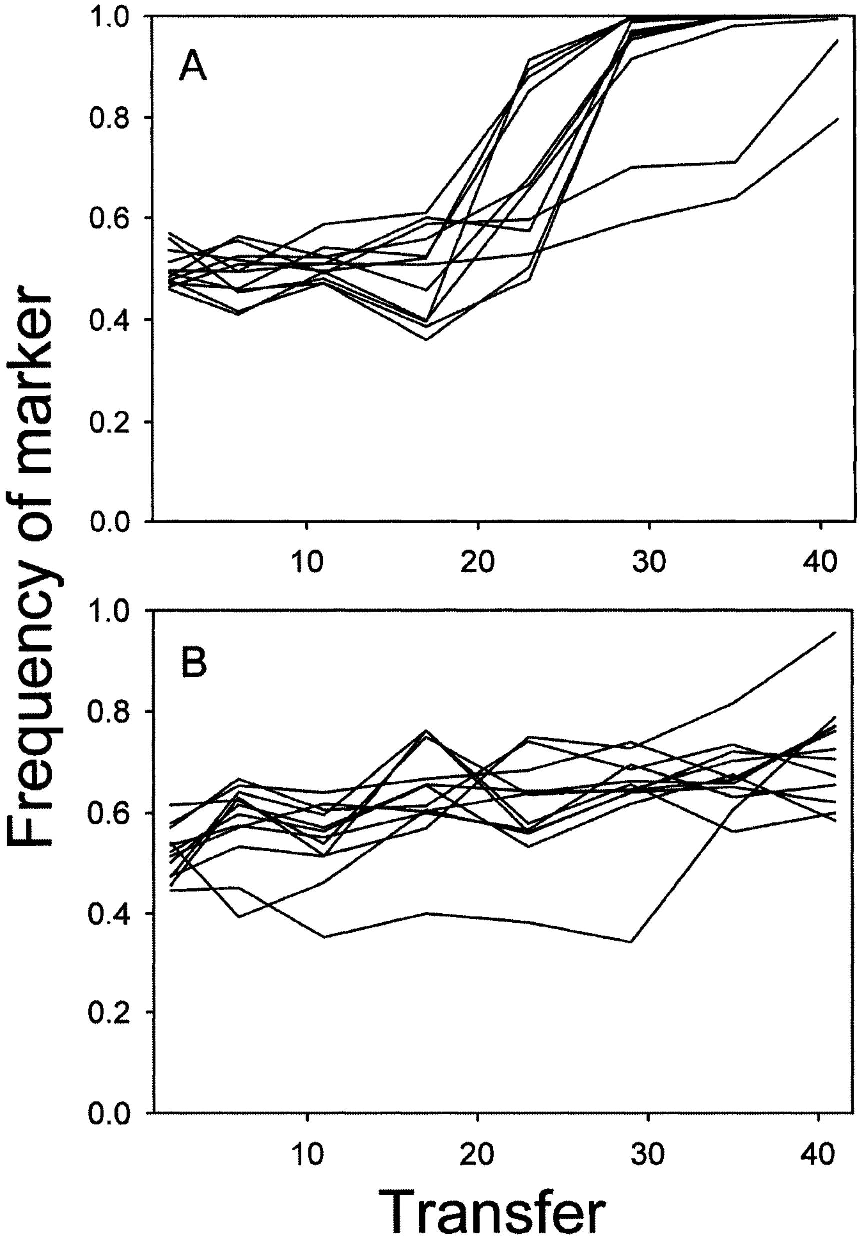

Figure 4: Fixation rate experiment. Solid lines show marker frequencies for each of 12 replicate lines selected in *A*, xylose and *B*, mannose. All lines converge to a frequency of one (and never zero) because the strain used as the denominator to calculate frequencies for a specific line is the one with the highest final frequency for that line.

Distribution of fitness among novel mutations

The distribution of selection coefficients in xylose and mannose among the 46 single-step mutants extracted from Kassen and Bataillon's (2006) library together with the ancestral strain is shown in figure 5. Our assay revealed 28 mutants with a selection coefficient significantly higher than that of the ancestor in xylose, against only two in mannose after correction for multiple comparisons (Dunnett's adjusted, df = 44, $P < 0.001$). This result is robust to the critical value used to assign significance, as using $\alpha = 0.01$ gave an additional six mutants beneficial in xylose, and only one in mannose. A Chi-square confirmed that 28/46 is significantly greater than 2/46 ($\chi^2 = 31$, df = 1, $P < 0.0001$). Taken together, these results suggest that there are many more beneficial mutations available to selection in xylose than mannose.

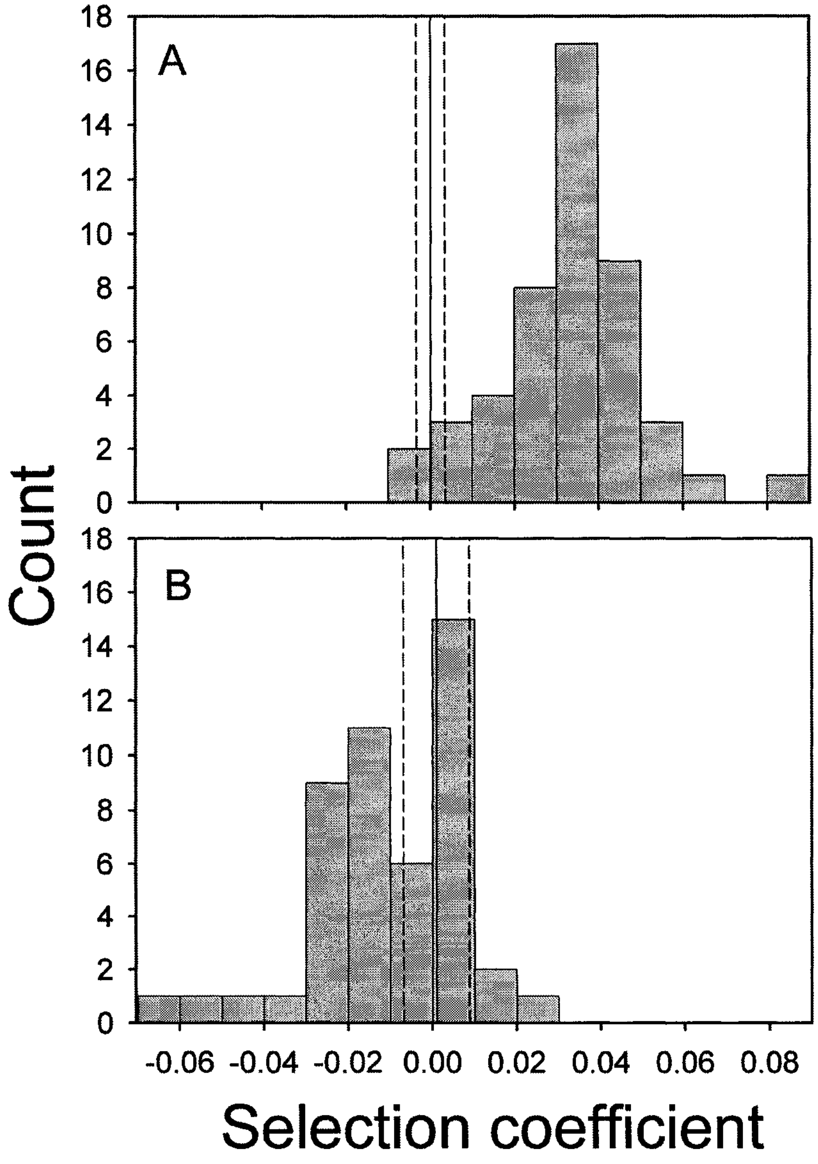

Figure 5: Frequency distribution of selection coefficients of the 46 mutants in xylose (*A*) and mannose (*B*). The solid vertical lines give the mean selection coefficient of the SBW25 ancestor when competed against the isogenic marked strain, with the dashed vertical lines indicating ± 95% confidence intervals of four replicates competitions.

Reversion lines

Selecting the two xylose-adapted lines in mannose for a further 25 transfers yielded divergent responses (Table 2). Replicate reversion lines derived from line A showed no response to selection on mannose ($F = 2.3$, df = 5, $P = 0.15$) while lines from line B showed a positive response to reverse selection ($F = 8.2$, df = 5, $P = 0.0069$). Selection coefficients in xylose show a similar pattern: reversion lines derived from line A showed no change ($F = 0.44$, df = 5, $P = 0.83$), while those derived from line B improved to xylose ($F = 5.8$, df = 5, $P = 0.018$). Note that for replicate reversions of line B the direct response to selection in mannose exceeded the correlated response in xylose, though both were in the same direction. These results indicate that antagonistic pleiotropy contributes little to the specificity of adaptation in xylose, at least in the short term.

Table 2: Reversion experiment. s is the selection coefficient per doubling in the assay environment.

	s in mannose	s in xylose
line	mean ± 1 S.E.M	mean ± 1 S.E.M
xylose specialist A	- 0.0021 ± 0.0016	0.8252 ± 0.1174
reversion A1	0.0384 ± 0.0087	1.2613 ± 0.7103
reversion A2	- 0.0628 ± 0.0548	1.0963 ± 0.4929
reversion A3	0.0534 ± 0.0517	0.7022 ± 0.0011
reversion A4	- 0.0165 ± 0.0684	0.7098 ± 0.6135
reversion A5	- 0.0509 ± 0.0020	0.7594 ± 0.0447
reversion A6	- 0.0346 ± 0.0354	0.9651 ± 0.5474
xylose specialist B	- 0.0096 ± 0.0058	0.6002 ± 0.0544
reversion B1	0.0770 ± 0.0199	0.7315 ± 0.4026
reversion B2	0.0227 ± 0.0251	0.4028 ± 0.0429
reversion B3	0.0678 ± 0.0470	0.8386 ± 0.1997
reversion B4	0.0329 ± 0.0059	1.2732 ± 0.0400
reversion B5	0.1208 ± 0.0102	0.5771 ± 0.0056
reversion B6	0.0064 ± 0.0095	1.1811 ± 0.1924

Discussion

Our experiment was designed to test the hypothesis that environmental heterogeneity in space at different scales affects the range of resource specialists that evolve in a population. We expected to observe a single specialist on the most productive resource when the environment was fine-grained or coarse-grained with unequal patch productivity, and a diverse collection of specialists, each adapted to a different resource, when the environment was coarse-grained with equal patch productivity. The most productive resource in our experiment was mannose (fig. 2), however we observed the evolution of mannose specialists only when it was the only resource available (fig. 3*B*) and when the environment was spatially coarse-grained and patch productivity was highly unequal (fig. 3*E*). In all other variable environments, we observed the evolution of specialists on xylose that paid a cost of adaptation on mannose (fig. 3*C*, *D*, *F*). This unexpected result is particularly striking in light of the significant direct response to selection in mannose alone (fig. 3*B*), which indicates that appropriate genetic variation for adaptation to mannose was available to selection.

The evolution of mannose-specialists when mannose is the sole carbon source (or xylose-specialists in xylose) is not hard to understand through simple selection theory: selection sorts among the available genetic variation introduced by mutation to produce adaptation to mannose (Lenski et al. 1991). The evolution of mannose-specialists in the spatially coarse-grained environment under unequal patch productivity also accords well with theory, although it has not been previously observed (see, for comparable experiments, Bell and Reboud 1997 and Cuevas et al. 2003). Mannose, being the more productive resource, contributes many more individuals to the total population than does xylose (see fig. 2). Any progress made by selection in improving fitness on xylose is therefore overwhelmed at each transfer by the immigration of types selected on mannose. Support for this interpretation comes from the calculation that the population size before transfer in the batch culture was about 32 times higher in mannose than in xylose, and that there were about 5.5 doublings per growth cycle in mannose against only one in xylose. Consequently, the effective population size, calculated as the minimum bottleneck size times the number of doublings (Lenski et al. 1991), was about 180 times larger in mannose than in xylose. Conversely, in populations from the spatially coarse-grained environment where patch productivity was equalized at each transfer (see methods), the effective population size in mannose was only 5.5 times greater than in xylose.

The evolution of xylose-specialists in the remaining three heterogeneous environments is the most striking and puzzling outcome of our experiment. Most often, selection in temporally variable environments leads to the evolution of generalists (reviewed in Kassen 2002; Buckling et al. 2006). In mixtures, the outcome of selection has usually been the evolution of specialization to the different resources available (Dykhuizen and Dean 2004; Friesen et al. 2004; Barrett et al. 2005). The outcome of selection in spatially coarse-grained environments has been less well-studied, the only two directly comparable experiment being that of Bell and Reboud (1997) who demonstrated the evolution of a diverse collection of niche specialists and that of Cuevas et al. (2003) who showed that specialists and generalists evolve at low and high migration rates, respectively. Our results stand in stark contrast to these experiments. Clearly, selection was constrained in some way that biases selection to favour adaptation to xylose rather than mannose.

The conventional interpretation for the evolution of constraints on the niche is clearly not appropriate here. Although adaptation to xylose was underlain by a cost of adaptation in mannose, the results of our reversion experiment suggest that this cost stems from mutation accumulation rather than antagonistic pleiotropy: a line previously adapted to xylose was able to adapt to mannose without compromising fitness in xylose (Table 2). Moreover, we did not observe a consistent within-line negative genetic correlation in fitness between xylose and mannose, with the exception of lines from the temporally varying treatment (line 1: $r^2 = 0.48$, $P = 0.0031$, slope ± S.E. = -6.69 ± 1.87; line 2: $r^2 = 0.53$, $P = 0.0013$, slope ± S.E. = -7.99 ± 1.99; see Egas et al. 2004; Dean 2005). Nevertheless, there appears to be little support for the notion that a fundamental functional constraint exists in the physiology of xylose and mannose use.

Rather, we suggest that the evolution of xylose specialists stems from constraints imposed by the mutational landscape available to selection in xylose and mannose. Under this view, beneficial mutations fix faster in xylose than mannose either because there are more of them or because they have, on average, larger beneficial effects. Our results lend support to this interpretation: lines selected in xylose fixed sooner than those in mannose (fig. 4) despite having a lower total population size and growth rate. Moreover the distribution of fitness effects among novel mutations, measured as the distribution of selection coefficients in competition against the ancestral strain, suggest that both the average beneficial effect of a new mutation and the number of beneficial mutations is larger in xylose than in mannose (fig. 5). Taken together, these two experiments represent strong evidence that the genetic variance ultimately responsible for adaptation is created at a faster rate in xylose than in mannose in our experiment.

The proximate cause underlying the broader mutational landscape in xylose when compared to mannose is unclear. Two non-mutually exclusive explanations seem plausible. First, the slow rate of growth in xylose constitutes a stressful environment that leads to elevated mutation rates (Tenaillon et al. 2004; Bennett et al. 1992) or elevated levels of expression of genetic variance (Hoffmann and Merilä 1999). Second, the ancestor may be substantially further off an adaptive peak in xylose than in mannose, implying that there is a larger variety of mutational targets for improving fitness in xylose than in mannose (Orr 2003, 2005).

It is conceivable that such constraints imposed by the availability of beneficial mutations are not unique to our experiment (Barton and Partridge 2000; Burch and Chao 2004; Blows and Hoffmann 2005; de Visser and Rozen 2005). Firstly, it has already observed that a high supply of beneficial mutations towards an allele promoted its fixation (Cooper et al. 2001). Furthermore, other experiments have noted differences in the rate of adaptation to different environments (Bennett et al 1992; Barrett and Bell 2006) or in different fitness components (Bull et al. 2004). In a particularly telling example, selection of an arbovirus in a temporally varying environment consisting of insect and mammalian host cells led to adaptation to the former but not the latter. This result was especially striking considering that the population size of the virus in insect cells was orders of magnitude below that of mammalian cells, and that populations selected only on mammalian cells were able to adapt to their host, indicating the availability of the adequate genetic variance (Zárate and Novella 2004). In light of our work, it would be interesting to test whether Zárate and Novella's observation might be explained by differences in the beneficial mutation supply rate between host cells.

Breaking the limits imposed by the supply of beneficial mutations (as opposed to standing genetic variance) has been invoked previously as an explanation for a range of biological phenomena such as the evolution of asexual organisms with elevated genomic mutation rates (Sniegowski et al. 1997; Taddei et al. 1997), ploidy levels (Zeyl et al. 2003), and sex (Barton and Charlesworth 1998; Colegrave 2002). Our results lend support to the idea that adaptation may often be constrained by the supply of beneficial mutations.

We have documented a particularly striking example of how the availability of beneficial mutations constrains the evolution of the ecological niche. This mechanism thus constitutes a third possible source of constraint on the evolution of niche breadth, the other two being costs of adaptation (Levins 1968) or trade-offs in the rate of evolution between specialists and generalists (Whitlock 1996). Given the absence of strong evidence for the commonness of costs of adaptation these alternative sources of constraints warrant further investigation.

Acknowledments

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Literature cited

Ackermann, M., and M. Doebeli. 2004. Evolution of niche width and adaptive diversification. Evolution 58:2599-2612.

Barrett, R. D. H., Maclean, R. C., and G. Bell. 2005. Experimental evolution of *Pseudomonas fluorescens* in simple and complex environments. American Naturalist 166:470-480.

Barrett, R. D. H., and G. Bell. 2006. The dynamics of diversification in evolving *Pseudomonas* populations. Evolution 60:484-490.

Barton, N. H., and B. Charlesworth. 1998. Why sex and recombination? Science 281:1986-1990.

Barton, N., and L. Partridge. 2000. Limits to natural selection. Bioessays 22:1075-1084.

Bell, G. 1990. The ecology and genetics of fitness in *Chlamydomonas*. I. Genotype-by-environment interaction among pure strains. Proceedings of the Royal Society of London. Series B, Biological Sciences 240:295-321.

Bell, G. 1997. Experimental evolution in *Chlamydomonas*. I. Short term selection in uniform and diverse environment. Heredity 78:490-497.

Bell, G., and X. Reboud. 1997. Experimental evolution in *Chlamydomonas*. V. Genetic variance in strongly contrasted environments. Heredity 78:498-506.

Bell, G., Lechowicz, M.J. , Appenzeller, A., Chandler, M., DeBlois, E., Jackson, L., Mackenzie, B., Preziosi, R., Schallenberg, M., and N. Tinker. 1993. The spatial structure of the environment. Oecologia 96:114-121.

Bell, G., Lechowicz, M. J., and M. J. Waterway. 2000. Environmental heterogeneity and species diversity of forest sedges. Journal of Ecology 88:67-87.

Bell, G., Lechowicz, M. J., and M. J. Waterway. 2006. The comparative evidence relating to functional and neutral interpretations of biological communities. Ecology 87:1378-1386.

Bennett, A. F., Lenski, R. E., and J. E. Mittler. 1992. Evolutionary adaptation to temperature. I. Fitness responses of *Escherichia coli* to changes in its thermal environment. Evolution 46:16-30.

Blows, M. W., and A. A. Hoffmann. 2005. A reassessment of genetic limits to evolutionary change. Ecology 86:1371-1384.

Bolnick, D. I. 2001. Intraspecific competition favours niche width expansion in *Drosophila melanogaster*. Nature 22:463-466.

Buckling, A., Brochhurst, M. A., Travisano, M., and P. B. Rainey. 2006. Experimental adaptation to high and low quality environments under different scales of temporal variation. Journal of Evolutionary Biology XX:XXX-XXX.

Bull, J. J., Badgett, M. R., Springman, R., and I. J. Molineux. 2004. Genome properties and the limits of adaptation in bacteriophages. Evolution 58:692-701.

Burch, C. L., and L. Chao. 2000. Evolvability of an RNA virus is determined by its mutational neighbourhood. Nature 206:625-628.

Colegrave, N. 2002. Sex releases the speed limit on evolution. Nature 420:664-666.

Cooper, V.S., and R.E. Lenski. 2000. The population genetics of ecological specialization in evolving *Escherichia coli* populations. Nature 407:736-739.

Cooper, V. S., Schneider, D., Blot, M., and R. E. Lenski. 2001. Mechanisms causing rapid and parallel losses of ribose catabolism in evolving populations of *Escherichia coli* B. Journal of Bacteriology 183:2834-2841.

Cuevas, J. M., Moya, M., and S. F. Elena. 2003. Evolution of RNA virus in spatially structured heterogeneous environments. Journal of Evolutionary Biology 16:456-466.

Day, T. 2000. Competition and the effect of spatial resource heterogeneity on evolutionary diversification. American Naturalist 155:790-803.

Dean, A. M. 2005. Protecting haploid polymorphism in temporally variable environments. Genetics 169:1147-1156.

Dempster, E. R. 1955. Maintenance of genetic heterogeneity. Cold Spring Harbour Symposium in Quantitative Biology 20:25-32.

Dykhuizen, D. E., and A. M. Dean. 2004. Evolution of specialists in an experimental microcosm. Genetics 167:2015-2026.

Egas, M., Dieckmann, U., and M. W. Sabelis. 2004. Evolution restricts the coexistence of specialists and generalists: the role of trade-off structure. American Naturalist 163:518-531.

Elena, S. F. 2002. Restrictions to RNA virus adaptation: An experimental approach. Antonie van Leeuwenhoek 81:135-142.

Friesen, M. L., Saxer, G., Travisano, M., and M. Doebeli. 2004. Experimental evidence for sympatric ecological diversification to frequency-dependent competition in *Escherichia coli.* Evolution 58: 245-260.

Futuyma, D.J., and G. Moreno. 1988. The evolution of ecological specialization. Annual Reviews of Ecology and Systematics 19:207-233.

Gillespie, J. 1974. The role of environmental grain in the maintenance of genetic variation. American Naturalist 108:831-836.

Hegreness, M., Shoresh, N., Hartl, D., and R. Kishony. 2006. An equivalence principle for the incorporation of favorable mutations in asexual populations. Science 311:16151-1617.

Hoffmann, A. A., and J. Merilä. 1999. Heritable variation and evolution under favourable and unfavourable conditions. Trends in Ecology and Evolution 14: 96-101.

Hubbell, S. P. 2001. The unified theory of biodiversity and biogeography. Princeton University Press, Princeton, N.J.

Jeanike, J. 1990. Host specialization in phytophagous insects. Annual Reviews of Ecology and Systematics 21:243-273.

Kassen, R., and G. Bell. 2000. The ecology and genetics of fitness in *Chlamydomonas*. X. The relationship between genetic correlation and genetic distance. Evolution 54: 425-432.

Kassen, R. 2002. The evolution of specialists, generalists, and the maintenance of diversity. Journal of Evolutionary Biology 15:173-190.

Kassen, R., and P. B. Rainey. 2004. The ecology and genetics of microbial diversity. Annual Reviews of Microbiology 58:207-231.

Kassen, R., and T. Bataillon. 2006. Distribution of fitness effects among beneficial mutations before selection in experimental populations of bacteria. Nature Genetics 38:484-488.

Lenski, R. E., Rose, M. R., Simpson, S. C., and S. C. Tadler. 1991. Long-term experimental evolution in *Escherichia coli*. I. Adaptation and divergence during 2,000 generations. American Naturalist 138:1315-1341.

Levene, H. 1953. Genetic equilibrium when more than one ecological niche is available. American Naturalist 87:331-333.

Levin, B. R., Perrot, V., and N. Walker. 2000. Compensatory mutations, antibiotic resistance and the population genetics of adaptive evolution in bacteria. Genetics 154:985-997.

Levins, R. 1968. Evolution in changing environments. Princeton University Press, Princeton.

Maclean, R. C. and Bell, G. 2002. Experimental adaptive radiation of *Pseudomonas.* American Naturalist 160:569-581.

Maclean, R. C., Bell, G, and P. B. Rainey. 2004. The evolution of pleiotropic fitness tradeoff in *Pseudomonas fluorescens.* Proceedings of the National Academy of Sciences of the USA 101:8072-8077.

Maynard Smith, J., and R. Hoekstra. 1980. Polymorphism in a varied environment: how robust are the models? Genetical Research 35:45-57.

Moore, F. B.-G., Rozen, D. E., and R. E. Lenski. 2000. Pervasive compensatory adaptation in *Escherichia coli.* Proceedings of the Royal Society of London B, Biological Sciences 267:515-522.

Orr, H. A. 2003. The distribution of fitness effects among beneficial mutations. Genetics 163:1519-1526.

Orr, H. A. 2005. The genetic theory of adaptation: a brief history. Nature Reviews Genetics 6:119-127.

Ostrowski, E. A., Rozen, D. E., and R. E. Lenski. 2005. Pleiotropic effects of beneficial mutations in *Escherichia coli.* Evolution 59:2343-2352.

Poon, A., and L. Chao. 2005. The rate of compensatory mutation in the DNA bacteriophage ΦX174. Genetics 170:989-999.

Rainey, P. B., and M. J. Bailey. 1996. Physical and genetic map of the *Pseudomonas fluorescens* SBW25 chromosome. Molecular Microbiology 19:521-533.

Robertson, A. 1959. The sampling variance of the genetic correlation coefficient. Biometrics 15:469-485.

Sniegowski, P. D., Gerrish, P. J., and R. E. Lenski. 1997. Evolution of high mutation rates in experimental populations of *E. coli*. Nature 387: 703-705.

Strobeck, C. 1975. Selection in fine-grained environment. American Naturalist 109:419-425.

Taddei, F., Radman, M., Maynard-Smith, J., Toupance, B., Gouyon, P. H., and B. Godelle. 1997. Role of mutator alleles in adaptive evolution. Nature 387:700-702.

Tenaillon, O., Denamur, E., and I. Matic. 2004. Evolutionary significance of stress-induced mutagenesis in bacteria. Trends in Microbiology 12:264-270.

Velicer, G. J. 1999. Pleiotropic effects of adaptation to a single carbon source for growth on alternative substrates. Applied and Environmental Microbiology 65:264-269.

De Visser, J. A. G. M., and D. E. Rozen. 2005. Limits to adaptation in asexuals. Journal of Evolutionary Biology 18:779-788.

Whitlock, M. C. 1996. The Red Queen beats the Jack-Of-All-Trades: the limitations on the evolution of phenotypic plasticity and niche breadth. American Naturalist 148:S65-S77.

Zárate, S., and I. S. Novella. 2004. Vesicular stomatitis virus evolution during alternation between persistent infection in insect cells and acute infection in mammalian cells is dominated by the persistence phase. Journal of Virology 78: 12236-12242.

Zeyl, C., Vanderford, T., and M. Carter. 2003. An evolutionary advantage of haploidy in large yeast populations. Science 299:555-558.

Zhan, J., Mundt, C. C., Hoffer, M. E., and B. A. McDonald. 2002. Local adaptation and effect of host genotype on the rate of pathogen evolution: an experimental test in a plant pathosystem. Journal of Evolutionary Biology 15:634-647.

Conclusion

It has traditionally been thought that niche breadth is limited by costs of adaptation emerging from antagonistic pleiotropy, where performance on one ecological condition is negatively correlated with performance on others because of direct trade-offs. The take-home message that pervades from the two experiments presented here is that evolutionary processes may prevent the expression of trade-offs. A more recent account of the evolution of limits to niches is that performance on uncommon conditions simply decays because of the inefficiency of natural selection to purge mutations disadvantageous to these environments. I take the results I presented as support for this latter idea, because they pointed towards two evolutionary factors that can prevent trade-offs from being reached.

Founders of the selection experiments were not well adapted to the laboratory environments and, for a trade-off to be expressed, traits involved in it must attain an evolutionary optimum (Fry 1996; Novak et al. 2006). I demonstrated the existence of two processes that may prevent reaching the trade-off line in phenotypic space within populations in the course of adaptation (see Figure 1): a positive relationship between selection strength and resource relative productivity in fine-grained heterogeneous environments (Chapter 1), and variation in the rate of beneficial mutations across resources (Chapter 2). The strength of natural selection increases with a resource's productivity and this will facilitate adaptation on productive resources and decline of performance on unproductive resources because of the accumulation of disadvantageous mutations (Kawecki et al. 1997). However, the experiments from Chapter 2 demonstrated that mutational parameters can override this effect of resource productivity. In this case, the main response to selection was adaptation to the resource to which the availability of beneficial mutations of large effect was the greatest, even though this resource was extremely unproductive.

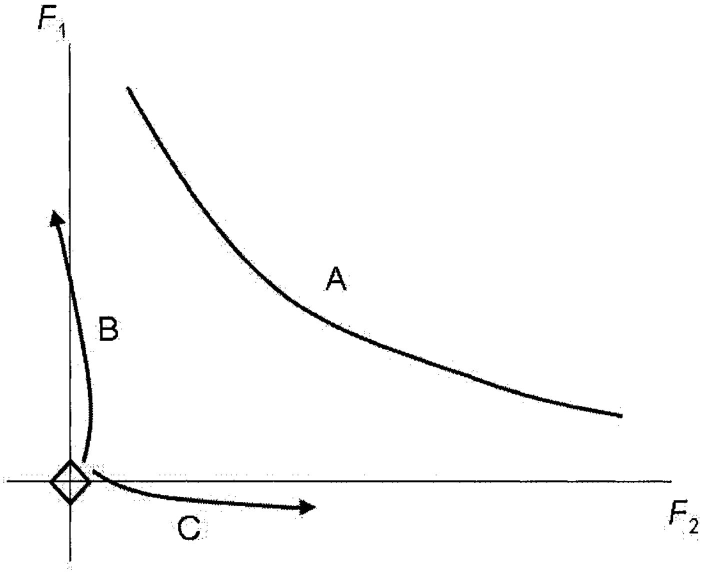

Figure 1: A fitness trade-off (*A*) on the phenotypic space of fitness *F* on resource *1* and *2*. A population colonizes an environment comprising both resources; its fitness starts at the origin (diamond) but can be prevented from reaching the trade-off function by two processes: the evolutionary trajectory *B* is dominated by the high productivity of resource *1* and is little influenced by resource *2*, while the alternative evolutionary trajectory *C* is dominated by a high beneficial mutation rate on *2* relative to *1*. Populations following these trajectories may never show the fitness trade-off between F_1 and F_2.

How can we reconcile these two outcomes? The productivity of the four resources used in the experiment that demonstrated the importance of resource productivity was never as low as that of xylose, for which beneficial mutations were available in abundance. The least productive resource of all four, mannose, was about 16 times more productive than xylose. Thus, the likely reconciliation is that beneficial mutations availability in mannose, glucose, mannitol and sorbitol is similar relative to that in xylose, and therefore it did not significantly direct selection in comparison to these resources' relative productivity.

Both experiments further revealed three interesting patterns arising across sympatric genotypes. Firstly, in Chapter 1, there was more the genotype-by-environment (G X E) interaction (a measure of genetic diversity) in two-resource environments than in single-resource environments. This is a direct demonstration that interactions between genotypes in mixtures favoured the maintenance of specialization on alternative resources, even though the main response to selection of populations was to adapt to the most productive resource.

Secondly, in that same experiment, the identity of the resources paired in mixtures predicted the G X E interaction between individuals within population. More specifically, I found a positive correlation between G X E in two-resource environments and G X E in the two resources they include (with fitness measured in the same pair of environments). This suggests that the amount of G X E that evolves within populations is a property of the pairs of resources. Although a similar phenomenon has been observed in ecological experiments (Bell 1992; Kassen and Bell 2000), it is the first time that it evolves *de novo*. There are two directions that future experiments could take: first, new experimental tests of the relationship between a measure of environmental variance between pairs of resources and diversity in mixtures of each pair, or second, explorations of lower levels of biological organization to understand the mechanistic basis of the presence and absence of antagonistic pleiotropy (Dean 1995; Lunzer et al. 2002). The later approach is likely to yield theories with greater predictive power in the sense that they will provide original testable predictions.

Thirdly, in the experiment comparing heterogeneous environments varying in space at different scales, the environment that varied through time was the only one to produce a replicable negative genetic correlation (i.e. a trade-off) across resources. This is unexpected in the light of classical theory for haploids (Maynard Smith 1998) and especially previous experimental results (Kassen 2002; Buckling et al. 2006). However, Dean (2005; see also Chesson 2000), provided probably the model most similar to this temporal variation treatment and he showed that a polymorphism can be maintained under these circumstances. This outcome of selection can thus be seen as support for a mathematical model rather than a peculiar result.

In conclusion, these experiments demonstrated the existence of two forces, one genetical and the other ecological, that can modulate adaptive evolution in heterogeneous environments. This suggests that even though trade-offs probably exist, they might never be attained. They should thus spur other experiments testing for the reach and generality of these forces.

Literature Cited

Buckling, A., Brochhurst, M. A., Travisano, M., and P. B. Rainey. 2006. Experimental adaptation to high and low quality environments under different scales of temporal variation. Journal of Evolutionary Biology XX:XXX-XXX.

Chesson, P. 2000. Mechanisms of maintenance of species diversity. Annual Reviews of Ecology and Systematics 31:343-366.

Dean, A. M. 1995. A molecular investigation of genotype-by-environment interactions. Genetics 139:19-33.

Dempster, E.R. 1955. Maintenance of genetic heterogeneity. Cold Spring Harbour Symposium in Quantitative Biology 20:25-32.

Fry, J. D. 1996. The evolution of host specialization: are trade-offs overrated? American Naturalist 148:S84-S107.

Kawecki, T.J., Barton, N.H., and J.D. Fry. 1997. Mutational collapse of fitness in marginal habitats and the evolution of ecological specialisation. Journal of Evolutionary Biology 10:407-429.

Lunzer, M., Natarajan, A., Dykhuizen, D. E., and A. M. Dean. 2002. Enzyme kinetics, substitutable resources and competition: from biochemistry to frequency-dependent selection in *lac*. Genetics 162:485-499.

Maynard Smith, J. 1998. Evolutionary Genetics. 2nd Edition. Oxford University Press, Oxford.

Novak, M., Pfeiffer, T., Lenski, R. E., Sauer, U., and S. Bonhoeffer. 2006. Experimental tests for an evolutionary trade-off between growth rate and yield in *E. coli*. American Naturalist 168:242-251.