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Evolution of a single niche specialist in variable environments

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The pattern (space versus time) and scale (relative to the lifetime of individuals) of environmental variation is thought to play a central role in governing the evolution of the ecological niche and the maintenance of genetic variance in fitness. To evaluate this idea, we serially propagated an initially genetically uniform population of the bacterium *Pseudomonas fluorescens* for a few hundred generations in environments that differed in both the pattern and scale at which two highly contrasted carbon substrates were experienced. We found that, contrary to expectations, populations often evolved into a single niche specialist adapted to the less-productive substrate in variable environments and that the genetic variance in fitness across different components of the environment was not generally higher in variable environments when compared with constant environments. We provide evidence to suggest that our results reflect a novel constraint on niche evolution imposed by the supply of beneficial mutations available to selection in variable environments.

Keywords: experimental evolution; environmental variation; productivity; fitness landscape; genetic diversity; *Pseudomonas fluorescens*

1. INTRODUCTION

The evolution of the ecological niche of a genotype or species, the range of conditions under which it can grow and reproduce, can be understood as the product of two opposing processes. The first is divergent natural selection caused by environmental variation that favours the evolution of ever-broader adaptation. The second results from the deleterious effects of alleles substituted by selection (antagonistic pleiotropy) or drift (mutation accumulation) which generate environment-specific costs of adaptation, thus preventing the evolution of a single universally superior type and, under certain conditions, facilitating the maintenance of diversity (Levins 1968).

The effectiveness of divergent selection at broadening niche width is thought to depend on the pattern (in time or space) and scale (relative to the lifetime of individuals) of environmental variation. Consider an environment composed of two patches or substrates that differ in the prevailing conditions of growth. Ecological generalists, individuals that maintain high fitness in both patches, are expected to evolve when the environment varies in time because a lineage is compelled to grow first in one patch and then the other (Hedrick 1986). Ecological specialists, types that are well-adapted to some patches but not others, are thought to evolve more readily when the environment varies in space because both patches, being simultaneously available, provide refuges from selection. However, the outcome of selection in spatially variable environments also depends on the scale at which the environment varies relative to the lifetime of individuals (Levins 1968). Environments that vary on time scales shorter than a single generation are said to be fine-grained, with selection expected to cause adaptation to the most productive patch and leading to the evolution of a narrowly adapted specialist (Strobeck 1975; Jasmin & Kassen 2007). Variation on scales longer than a generation is termed coarse-grained and, provided the number of individuals supported by each patch is roughly equal, is expected to be more effective at promoting the evolution of a diversity of specialized types because directional selection within each patch is preserved through their segregation in space (Levene 1953; Dempster 1955; Day 2000).

Here we evaluate the importance of the pattern and scale of environmental variation for the evolution of niche width and the maintenance of diversity by following the fate of genetic variation arising through mutation in evolving populations of the bacterium *Pseudomonas fluorescens*. In our experiments, four variable environments were created by varying the pattern and scale at which a population experiences two contrasted carbon substrates, xylose and mannose (figure 1). We expected that spatially coarse-grained environments would maintain more genetic variation in fitness across substrates, measured as the inconsistency component of the genotype-by-environment interaction, than spatially fine-grained environments composed of the same substrates. Our results, however, were surprising: we often observed the evolution of a single specialist adapted to the substrate that made the smallest contribution to population growth (i.e. the least productive). We interpret these results as consistent with the idea that the mutational landscape available to selection constrains adaptive evolution in variable environments by controlling the supply, and so the fitness effect, of beneficial mutations.

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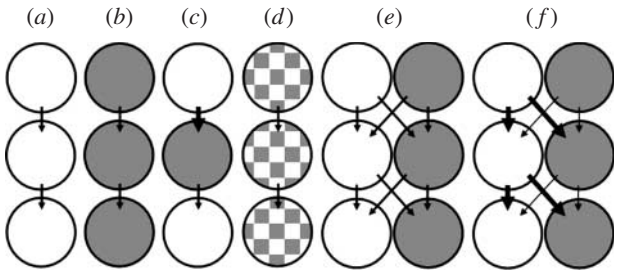

Figure 1. Selection environments. Circles represent microwells containing either xylose (white) or mannose (grey). Arrows represent a transfer; bold arrows represent larger volumes transferred to compensate for productivity differences. (*a*,*b*) Constant environments: aliquots transferred to the same substrate, either (*a*) xylose or (*b*) mannose; (*c*) temporally varying environment: alternation between xylose and mannose; (*d*) fine-grained environment: mixture of mannose and xylose; (*e*) spatially coarse-grained environment with unequal substrate productivity: the contribution of each substrate to the total population is a function of that substrate's productivity; (*f*) spatially coarse-grained environment with equal substrate productivity: the fraction of individuals contributed by each carbon source to the total population is the same.

2. MATERIAL AND METHODS

(a) *Founding strains and growth conditions*

The founder was a single colony of the soil bacterium *P. fluorescens* SBW25::*lacZ*, which is isogenic to the wild-type SBW25 strain used in previous experiments (Rainey & Bailey 1996; Jasmin & Kassen 2007), save the insertion of a promoterless *lacZ* allele in a phage region (X.-X. Zhang 2006, personal communication). Colonies possessing the *lacZ* insertion turn blue when plated on agar supplemented with 40 mg l^{-1} 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) and so can be readily distinguished from the wild-type strain which is yellow. The ancestral and SBW25 clones were frozen in 16% (v/v) glycerol at −80°C. The selection medium consisted of M9 minimal salts (1 g l^{-1} NH_4Cl, 3 g l^{-1} KH_2PO_4, 0.5 g l^{-1} NaCl and 6.8 g l^{-1} Na_2HPO_4) supplemented with 15 mg l^{-1} $CaCl_2$ and 0.5 g l^{-1} $MgSO_4$, and a source of carbon provided at a concentration of 1.70×10^{-3} M. Monosaccharides were mannose (306 mg l^{-1}), xylose (255 mg l^{-1}) or a fine-grained mixture of both (306 mg l^{-1} mannose plus 255 mg l^{-1} xylose). All populations were cultured on 24-well plates (Cellstar; Greiner Bio-One, Frickenhausen, Germany), with 2 ml of media in each well, in an orbital shaker (150*g*) at 28°C.

(b) *Main selection experiment*

The experiment consisted of two replicates of each of six selection regimes that differed in the pattern and scale at which the two carbon sources, xylose and mannose, were provided (figure 1). Aliquots of exponentially growing cultures were transferred every 12 hours for 101 transfers. We equalized the effective population size (N_e) across treatments to approximately 6×10^6 cells by manipulating the volume of media (and so the number of cells) transferred. Here, N_e is the harmonic mean of the minimum and maximum population sizes reached in a growth cycle. We estimated population density at the end of a growth cycle by converting the optical density of cultures measured at 660 nm (spectrophotometer EL_X-800; Bio-Tek Instruments, Inc., Winooski, VT, USA) into cell density using a standard curve. Growth rates in mannose are substantially faster than in xylose (5.5 doublings in mannose versus 1 in xylose) which means that lines selected in mannose alone evolved for approximately 550 generations while those in xylose alone evolved for approximately 100 generations.

(c) *Fitness assay*

Fitness of evolved lines was estimated from 16 colonies isolated from each line and competed separately against the ancestral genotype lacking the *lacZ* insertion in both xylose and mannose. All strains were first acclimated from frozen cultures on the substrate of competition for two growth cycles. Evolved and ancestral strains were then mixed at a 1 : 1 ratio (equal cell densities) for the competition. Strains were allowed to compete for four growth cycles, with estimates of the relative frequency of the two types assayed after the first (initial frequency after 12 hours) and fourth (final frequency after 48 hours) growth cycles by plating on agar plates containing M9 minimal salts supplemented with X-Gal. We calculated the relative fitness, *w*, a measure of the rate at which the frequency of an evolved genotype changes relative to its ancestor, as

$$w = 1 + \frac{\ln(f_E)_{final} - \ln(f_E)_{initial}}{\text{doublings}}, \qquad (2.1)$$

where f_E is the frequency of an evolved genotype relative to the ancestor and *doublings* refers to the number of doublings by the ancestor in either mannose or xylose over the three growth cycles of competition. We assessed the response to selection in two ways. First, we conducted a factorial ANOVA with selection environment and assay substrate as main effects; a significant interaction term indicates evolutionary divergence of populations due to selection in different environments. We then assessed the significance of the direct and correlated responses for each selection line on each substrate using two-tailed *t*-tests. This procedure gives a total of 24 tests (=six treatments×two replicate lines×two assay substrates) so we adjusted significance levels using a Bonferroni correction ($\alpha=0.05/24=0.002$). All statistical analyses were performed in JMP (SAS Institute, Inc.). Note that in both cases the error term is likely to be conservative as it conflates true experimental error with genetic variation among the 16 isolates from each selection treatment.

Obtaining separate fitness estimates for each of the 16 colonies from a selection line allows us to estimate the quantity of diversity supported by each environment. We estimated diversity as the inconsistency component of the genotype-by-environment interaction within each selection line, which measures the amount of crossing in the norms of reactions of the 16 genotypes or, more formally, the lack of correlation in fitness among genotypes in a pair of environments (Robertson 1959; see also Bell 1990; Barrett *et al.* 2005).

(d) *The distribution of fitness among novel mutations*

We estimated the distribution of relative fitness among independently isolated, single-step mutants using the *P. fluorescens* SBW25 mutant library reported in Kassen & Bataillon (2006), who provide detailed description of methods. 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 Luria–Bertrani

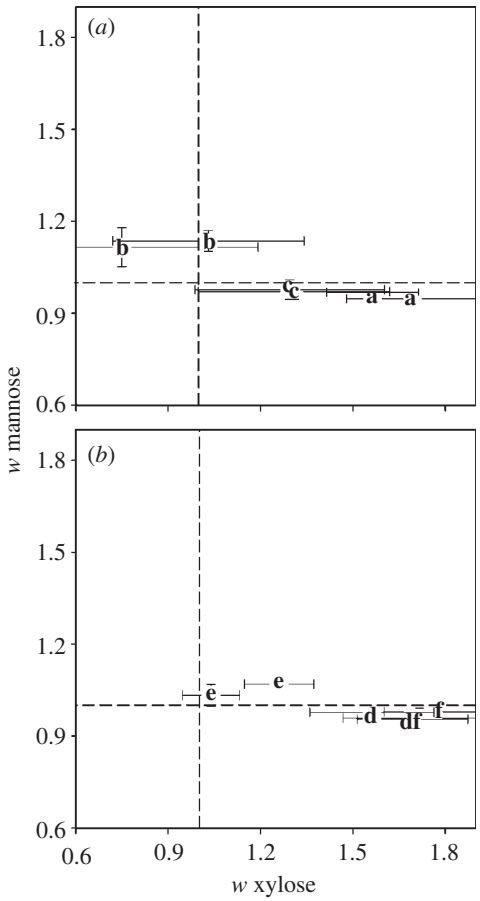

Figure 2. Mean relative fitness (per doubling) of genotypes isolated from each of two replicate lines selected in (*a*) constant xylose, **a**; constant mannose, **b**; temporally varying environment, **c**, and (*b*) the spatially fine-grained environment, **d**, and spatially coarse-grained environments with unequal, **e**, or equal, **f**, substrate productivity. Data points represent average ± s.d. of 16 colonies tested for each population. Error bars not visible are hidden by a symbol.

medium, the same medium used for antibiotic selection, and estimated the fitness of each relative to their antibiotic-sensitive ancestor (SBW25::*lacZ*), in both xylose and mannose. Competition experiments were performed as described previously. Two replicate estimates of relative fitness were obtained for each mutant strain. Note that this library was created from the same strain used to found the selection lines in our experiment and so provides some direct insight into the mutational landscape available to this strain.

3. RESULTS

(a) *Response to selection*

Figure 2 shows the response to selection in xylose and mannose alone for all evolved populations. The relative fitness of an evolved genotype assayed on xylose or mannose depends on the selection environment from which it was isolated, as demonstrated by a significant selection environment × assay substrate interaction (table 1). We thus further analysed these data using multiple comparisons (one-sample *t*-tests testing for relative fitness different from one, $n=16$). Replicate lines selected in both constant environments adapted to their respective substrate (figure 2*a*; xylose: $t=13$ and 15, $p<0.0001$; mannose: $t=7.3$ and 16, $p<0.0001$), although correlated responses to selection in the alternative substrates were variable, as indicated by the significance of the assay substrate × line (selection environment) term in the ANOVA reported in table 1. Lines selected on xylose paid a cost of adaptation in mannose ($t=-7.3$ and -20, $p<0.0001$), whereas mannose-selected lines showed no consistent correlated response in xylose ($t=-2.3$, $p=0.039$ and $t=0.69$, $p=0.70$).

Table 1. ANOVA of the relative fitness for 16 genotypes isolated from each selection line. (The variance explained by the model (R^2) is 75%.)

source	d.f.	sum of squares	*F*	*p*
selection environment	5	5.92	9.60	0.008
assay substrate	1	14.0	140	<0.0001
line (selection)[a]	6	0.734	1.23	0.4
selection × substrate	5	12.4	24.9	0.0006
substrate × line (selection)	6	0.600	3.22	0.004
error	360	11.2	mean square = 0.0311	

[a] Line nested within selection environment was a random factor while all other factors were fixed.

The response to selection in the variable environments was unexpected. We observed adaptation to xylose (figure 2*a*,*b*; all tests: $t>3.7$, $p<0.001$) but not mannose in all treatments save one, the spatially coarse-grained treatment with unequal productivity, where the response for both replicate lines was positive in mannose (figure 2*b*; $t>3.7$, $p<0.001$) but not formally significant in xylose (one line's response was significant with $p<0.001$ but the other was not: $t=1.7$, $p=0.11$). Adaptation to xylose in the other three variable environments was accompanied by a statistically significant cost of adaptation in mannose for all lines ($t>-3.73$, $p<0.001$) but two, which became marginally significant after correcting for multiple comparisons (one replicate line in the spatially coarse-grained environment with equal productivity: figure 2*b*; $t=-3.5$, $p<0.01$ and another in the temporally variable environment: figure 2*a*; $t=-2.9$, $p<0.05$).

Table 2 reports the inconsistency component of the genotype-by-environment interaction for all treatments. Recall that the inconsistency is a measure of the amount of crossing in reaction norms for fitness in mannose and xylose (Robertson 1959) and, for populations in variable environments, estimates the degree to which genotypes specialize on alternative substrates. Contrary to expectations, inconsistency was largest in the temporal variation treatment and there was little effect of the scale of environmental variation on the quantity of diversity maintained in spatially variable environments. Lines selected solely in mannose also showed a surprisingly high inconsistency component, perhaps because one population was sampled in the course of a selective sweep, although the mean inconsistency for these lines was not significantly larger than 0 ($p=0.11$ from one-tailed *t*-test with $n=4$). This result suggests that diversity did not greatly exceed its initial value in the founding population. In the five remaining treatments, the inconsistency was significantly greater than 0 ($p<0.05$).

(b) *Distribution of fitness among novel mutations*

The distribution of relative fitness in xylose and mannose among the 46 single-step mutants extracted

Table 2. The average inconsistency component of the genotype-by-environment interaction (±s.d.) 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, $\alpha = 0.05$.)

treatment	group	mean ± 1 s.d. ($\times 10^{-3}$)
spatially fine-grained	b	2.5 ± 0.4
spatially coarse-grained, equal productivity	b	3.3 ± 0.6
spatially coarse-grained, unequal productivity	b	4.3 ± 0.4
xylose	b	2.5 ± 0.4
mannose	ab	8.8 ± 4.6
temporal variation	a	15.9 ± 0.9

from Kassen & Bataillon's (2006) library together with the ancestral strain is shown in figure 3. Our assay revealed 28 mutants in xylose with a relative fitness significantly higher than that of the ancestor but only two in mannose after correction for multiple comparisons (Dunnett's test, $p < 0.001$), and 28/46 is significantly greater than 2/46 ($\chi^2 = 31$, $p < 0.0001$). This result is robust to the critical value used to assign significance: using $\alpha = 0.01$ gave an additional six mutants beneficial in xylose and only one in mannose. These results suggest that there are many more beneficial mutations available to selection in xylose than mannose, implying that the average fitness effect of the mutations substituted in xylose is likely to be greater than in mannose.

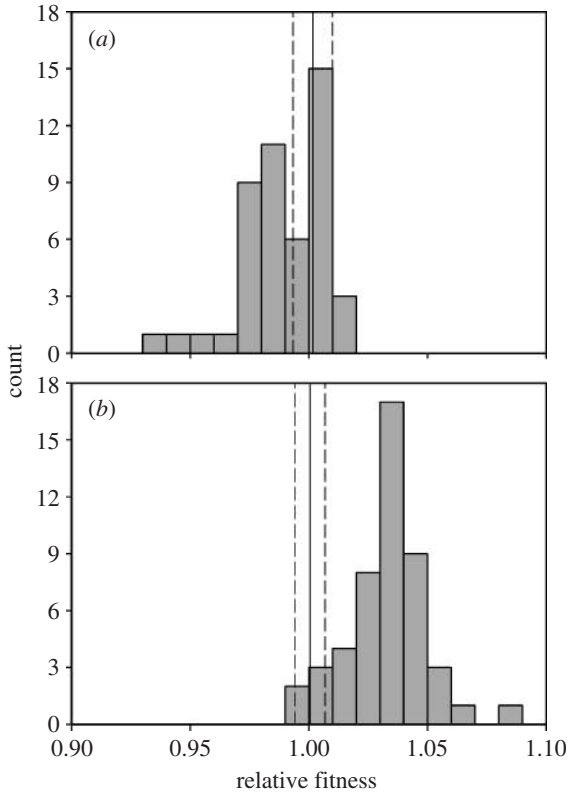

Figure 3. Frequency distribution of relative fitness for the 46 mutants in (*a*) mannose and (*b*) xylose. The solid vertical lines give the mean relative fitness of the SBW25 ancestor when competed against the isogenic marked strain, with the dashed vertical lines indicating ±95% CIs of four replicate competitions.

4. DISCUSSION

Our experiment was designed to test the hypothesis that the pattern and scale of environmental variation controls the evolution of niche specialization and diversity. When the relative productivity of different patches in a variable environment is unequal (figure 1*d*,*e*), selection should lead to the evolution of a single niche specialist adapted to the most productive patch. In turn, a range of niche specialists, each adapted to different patches of a coarse-grained spatially variable environment, can be maintained by divergent natural selection when the patches contribute roughly the same numbers of individuals to the total population (figure 1*f*).

(a) *Response to selection*

In our experiment, the most productive substrate was mannose and, as expected, we observed the evolution of mannose-specialists when the environment was spatially coarse-grained and the productivity of the two substrates was not experimentally equalized (figure 2*b*). This result accords well with theory (Hedrick 1986) but has not been observed previously (see, for comparable experiments, Bell 1997; Bell & Reboud 1997; Cuevas *et al.* 2003). Mannose contributes many more individuals to the total population than does xylose in this environment (there were approx. 5.5 doublings per growth cycle in mannose against only one in xylose). Mutants carrying a mutation beneficial in xylose are therefore overwhelmed by the immigration of types having an advantage in mannose.

In all other variable environments, however, we observed the evolution of specialists on xylose, the less-productive substrate, that also paid a cost of adaptation on mannose (figure 2*a*,*b*). This result was especially surprising given that selection in mannose alone is capable of generating adaptation to mannose and that, in previous microbial evolutionary experiments, selection in temporally varying environments almost invariably lead to the evolution of broadly adapted generalists (reviewed by Kassen 2002). We suggest that this outcome arises from differences in the effective supply of beneficial mutations available to selection on each substrate. In population genetics models, the mutation supply rate is a product of the effective population size and the mutation rate (De Visser *et al.* 1999). In our experiment we made an effort to equalize the effective population size in mannose and xylose in the temporally varying and the coarse-grained with equal substrate productivity environments (see §2), which should have the effect of minimizing any differences among substrates in the effective supply of mutations provided that mutation supply rates remain roughly constant across substrates. Nevertheless, the results reported in figure 3 suggest that this is not the case: the number of single-step beneficial mutations available to selection is much larger in xylose than in mannose, implying that the average fitness effect of a beneficial mutation substituted by selection will on average be much larger in xylose than

in mannose (Orr 2003). This difference in the availability and size of beneficial mutations would favour the evolution of xylose specialists over mannose specialists in our experiment.

To evaluate this interpretation further, we asked whether we could predict the outcome of selection in variable environments from knowledge of the response to selection in xylose and mannose alone. If adaptation in variable environments proceeds through the substitution of beneficial mutations with patch-specific effects then we should be able to predict the fitness of lines selected in variable environments from the fitness effect of mutations substituted in constant environments. Here we calculate the expected mean fitness of a xylose or mannose specialist in each variable environment using the responses to selection of populations evolved on each constant environment. Note that the appropriate mean to calculate depends on the nature of environmental variation. When substrate use is sequential, as it must be in a temporally varying environment, the appropriate estimate of fitness is the geometric mean across environments (Hedrick 1986). Mannose and xylose are also used sequentially in the fine-grained environment, indicating that the geometric mean is appropriate here as well (cell density in the fine-grained environment is similar to those observed in mannose over the first 8 hours of the 12 hours growth cycle and exceeds that in mannose during the last 4 hours, suggesting that the population uses predominantly xylose during the 4 hours before transfer; J.-N. Jasmin 2006, unpublished data). In our spatially variable environments, the relevant measure of fitness is the arithmetic mean fitness (Levins 1968), weighted by the relative contribution of each patch to the total population. We estimated the relative contribution of each substrate in our experiment from the growth rate of the ancestor on each substrate as 0.854 and 0.146 in mannose and xylose, respectively (for a total of one during a growth cycle). Note also that our transfer procedure ensured that the relative productivity of each substrate was identical for the temporally varying environment and the spatially coarse-grained environment with equal substrate productivity. Our results, reported in figure 4, indicate that the expected fitness of xylose specialists evolving in a variable environment is larger than that of mannose specialists under all kinds of variable environment except the spatially coarse-grained environment with unequal substrate productivity (for which $p=0.17$, one-tailed t-test, $n=4$). These results qualitatively match those observed in our selection experiment (see figure 2) and provide further support for the idea that the supply of mutations with large beneficial effects was higher in xylose than mannose, the result being the evolution of xylose specialists following selection in variable environments.

Taken together, our results suggest that the outcome of selection in variable environments can be constrained by the supply of beneficial mutations available to selection, also known as the mutational landscape (Gillespie 1991). Constraints imposed by the availability of beneficial mutations are not unique to our experiment (see Burch & Chao 2000; Blows & Hoffmann 2005; De Visser & Rozen 2005). For example, Cooper *et al.* (2001) observed that a high supply of beneficial mutations towards an allele promoted its fixation.

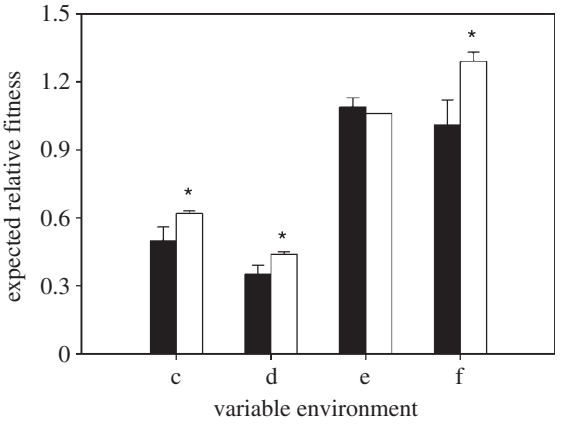

Figure 4. Mean expected relative fitness (±s.d.) in each variable environment estimated from the mannose (filled bars) and xylose (open bars) specialist populations selected in constant environments. Variable environments are as in figure 1: (c) temporally varying, (d) fine-grained and spatially coarse-grained with (e) unequal or (f) equal substrate productivity. Asterisks indicate significant differences contrasting expected relative fitness of xylose and mannose specialists for each environment (one-tailed t-tests, $p<0.05$, $n=4$ for each test).

Other experiments have also noted differences in the rate of adaptation to different environments (Barrett *et al.* 2005) or for different fitness components (Bull *et al.* 2004), consistent with the idea of variation in the mutational landscape constraining the outcome of selection. These empirical findings suggest that knowledge of the mutational landscape is integral to predicting the outcome of selection in multiple environments or on multiple characters.

The proximate causes underlying the differences in the mutational landscape for xylose and mannose remain unclear. Two non-mutually exclusive explanations seem plausible. First, the slow rate of growth in xylose may constitute a stressful condition that leads to elevated mutation rates (Tenaillon *et al.* 2004) or elevated levels of expression of genetic variance (Hoffmann & Merilä 1999). Second, the ancestor may be substantially further off an adaptive peak in xylose than in mannose, implying that there is a larger mutational target for improving fitness in xylose than in mannose (Orr 2003). This latter hypothesis is tentatively supported by our assay of single-step mutants resistant to naladixic acid that revealed positive pleiotropic effects that were on average larger in xylose than in mannose (figure 3).

(b) *Diversity*

As a general rule, diversity is more readily supported in heterogeneous environments than in homogeneous ones (Kassen 2002). Nevertheless, we observed no higher levels of diversity, on average, in variable environments and our interpretation of the importance of the mutational landscape in governing the outcome of selection under these conditions explains why. Despite the opportunity for strong divergent selection generating different niche specialists (figure 2*a*), the bias in the mutational landscape ensured that selection was more often effectively directional, leading to the evolution of a single niche specialist on either mannose or xylose.

Interestingly, and in contrast with most theory (Hedrick 1986; but see Dean 2005) and experiment (Kassen 2002), the temporally variable environment supported the highest level of genetic diversity (measured by the inconsistency component of the genotype-by-environment interaction; table 2) where we expected to observe a single, broadly adapted generalist. This result appears to stem from the cost of adaptation on mannose associated with adaptation to xylose (figure 2). Other experiments have similarly observed the evolution of specialization underlain by strong costs of adaptation in temporally varying environments. For 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 is especially striking considering that the viral population size in insect cells was orders of magnitude below that in mammalian cells, and that populations can rapidly adapt to mammalian cells (Zárate & Novella 2004; see also Ciota *et al.* 2007).

(c) *Summary*

In variable environments composed of two substrates with a more than fivefold difference in productivity, we observed the evolution of specialization to the least productive substrate. This led us to argue that we have documented an example of how the effective supply of beneficial mutations, which depends both on the nature of the mutational landscape and the relative productivity of different patches, may constrain the evolution of the ecological niche. This mechanism thus constitutes a third possible source of constraint on the evolution of niche width, the other two being costs of adaptation (Levins 1968) and trade-offs in the rate of adaptation between specialists and generalists (Whitlock 1996). Given the absence of strong evidence for costs of adaptation in field and laboratory experiments, these alternative sources of constraints warrant further investigation.

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