How does resource supply affect evolutionary diversification?

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The availability of different resources in the environment can affect the outcomes of evolutionary diversification. A unimodal distribution of diversity with resource supply has been widely observed and explained previously in the context of selection acting in a spatially heterogeneous environment. Here, we propose an alternative mechanism to explain the relationship between resource supply and diversification that is based on selection for exploitation of different resources. To test this mechanism, we conducted a selection experiment using the bacterium Pseudomonas fluorescens in spatially homogeneous environments over a wide range of resource supply rates. Our results show that niche diversification peaks at intermediate levels of resource availability. We suggest that this unimodal relationship is due to evolutionary diversification that is driven by competition for resources but constrained by the ecological opportunity represented by different resource types. These processes may underlie some general patterns of diversity, including latitudinal gradients in species richness and the effects of anthropogenic enrichment of the environment.

Keywords: Pseudomonas fluorescens; selection experiment; adaptive diversification; resource supply; diversity; productivity

1. INTRODUCTION

A central aim in evolutionary biology is to understand which factors explain patterns of adaptation and diversity. For example, diversity is greater in some environments than others, and some species are narrowly specialized while others are generalists (Futuyma & Moreno 1988; Gaston 2000). One factor that is often cited in explaining these patterns is the availability of resources in the environment (Tilman 1982; Abramsky & Rosenzweig 1984; Rosenzweig 1995). Although any general relationship remains unclear (Abrams 1995; Rosenzweig 1995), a unimodal distribution of species richness with resource supply has been observed at a range of spatial scales (Abrams 1995; Rosenzweig 1995). Several mechanisms have been suggested to account for these observations (Rosenzweig 1995), but experimental tests have proved problematic owing to the time-scale typically associated with evolutionary diversifications.

Microbial systems offer a way to address these questions experimentally. Micro-organisms are small and reproduce rapidly; hence, evolution can be observed in real time, in large populations, and in workable laboratory conditions (Dykhuizen 1992; Elena & Lenski 2003; Jessup et al. 2004). Pseudomonas fluorescens is a bacterium that is being used increasingly to study diversity in the laboratory (e.g. Rainey & Travisano 1998; Buckling et al. 2003; MacLean & Bell 2003; Brockhurst et al. 2004). Populations that are initially uniform will rapidly diversify into a number of specialist genotypes when selected in spatially structured microcosms (static tubes of growth media). The different genotypes that emerge are adapted to different spatial niches within the environment (Rainey & Travisano 1998) and are identified by their different colony morphologies. If spatial structure is removed, by constantly shaking the tubes, diversity is quickly lost since there is no longer a range of distinct niches. Kassen et al. (2000, 2004) experimentally manipulated nutrient concentration in static microcosms and found that morphological diversity peaks at intermediate levels of resource supply. This pattern was explained by the effects of nutrient concentration on the relative productivities of alternative spatial niches in the heterogeneous environment (Kassen et al. 2000).

However, these results may not provide a general explanation for the link between diversity and resource supply that is widely observed in natural communities. In these experiments, the same phenotypes emerge repeatedly and diversification is based on adaptation to spatially discrete niches (Kassen et al. 2004). While spatial structure is an important component of natural environments, most species do not occupy spatially distinct niches and instead exploit a subset from a wide range of simultaneously available resources. For example, the diversification in beak morphology among Darwin’s finches is explained by parallel variation in the size and shape of food resources (Lack 1947; Grant 1986). Selection based on exploitation of available resources may be a more general feature of evolutionary diversification than the effects of spatial heterogeneity.

If this is the case, then we expect the outcomes of diversification to vary with the availability of different resources, even in a spatially homogeneous environment. Bacteria in spatially uniform conditions can diversify to specialize on particular substrates if a range of different resources are available (Friesen et al. 2004; Barrett et al. 2005; Tyerman et al. 2005; Barrett & Bell 2006). For example, if P. fluorescens is selected in a complex environment containing multiple carbon sources, the

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population diversifies into a number of phenotypes with slightly different niches, as defined by their performance on different carbon sources (Barrett et al. 2005; Barrett & Bell 2006). In this paper, we use experimental populations of P. fluorescens to examine the role of resource availability in adaptive diversification in spatially homogeneous environments.

We compare the outcomes of long-term evolution in replicate populations selected in environments that differed only in resource supply rate. We used similar complex environments to that of Barrett et al. (2005) but at several different resource supply rates. Following selection, adaptation and phenotypic diversity within each population were quantified from variation in growth rates between individual genotypes on each substrate within the complex environment. By measuring the degree to which bacteria diversify and adapt to different subsets of available resources (different niches), we can test critically the role of resource supply rate in the evolution of niche breadth and diversity.

2. MATERIAL AND METHODS

(a) Selection experiment

A clonal isolate of P. fluorescens SBW25 was used to found all selection lines, so that all populations were initially isogenic. The ancestral strain is stored at −80°C in 50% v:v glycerol. Selection was carried out in 28 ml glass universal tubes containing liquid media at 28°C under shaken conditions. This constitutes a spatially homogeneous environment. The liquid media in each tube comprised 6 ml of M9 salt solution (NH4Cl 1 g l⁻¹, Na2HPO4 6 g l⁻¹, KH2PO4 3 g l⁻¹ and NaCl 0.5 g l⁻¹) plus an equal concentration (g l⁻¹) of each of the four carbon sources. Carbon substrates were selected that have previously been shown to support growth and adaptation of P. fluorescens SBW25 (MacLean et al. 2004; Barrett et al. 2005), i.e. acetic acid, glycerol, malic acid and succinic acid. Thus, resource supply rate can be easily manipulated by changing the total concentration of carbon substrates in liquid media. Based on pilot work, we chose appropriate concentrations so that population density varied between selection environments. Population density was measured following growth over 48 h by optical density at 600 nm using a Jenway 6300 Spectrophotometer. We initiated selection lines in each of six selection environments, each with a specific resource supply rate. Resource supply was increased twofold between selection environments from the lowest level, so that lines were selected at the following concentrations: 0.009375; 0.01875; 0.0375; 0.075; 0.15; and 0.3 g l⁻¹. Eight replicate lines were selected at each level of resource supply for approximately 500 generations by transferring 60 µl from each 6 ml culture to fresh media every 2 days for 50 transfers. Following selection, all lines were frozen at −80°C in 50% v:v glycerol.

(b) Phenotypic assays

Patterns of diversity and specialization were determined from variation in growth rate between individual genotypes on each of the different carbon sources. We measured the growth rate of 10 genotypes from each population on each of the four substrates. To isolate distinct genotypes, each population was reconditioned overnight in standard growth medium (King’s medium B (KB): proteose peptone 20 g l⁻¹, glycerol 12 g l⁻¹, K2HPO4 1.5 g l⁻¹, MgSO4·7H2O 1.5 g l⁻¹) at 28°C under shaken conditions. Two replicate populations of the ancestral clone were also reconditioned. Cultures were then diluted and spread on agar plates. Ten colonies were picked at random from each population, constituting 10 genotypes. The growth of each genotype was then assayed twice on all four substrates independently at a concentration of 0.15 g l⁻¹. The same concentration was used in all assays to allow comparisons of growth rates across selection environments.

To ensure that cells in all the trials were initially at equivalent physiological states, each genotype was first grown in standard KB for 2 days and then starved for 2 h by transferring it to M9 salt solution. To begin the assay, 20 µl of starved cells (approx. 10⁵ viable cells) were then transferred to individual wells of 96-well plates containing 180 µl of the appropriate assay media (M9 salt solution plus 0.15 g l⁻¹ of one of the four carbon substrates). The number of assays involved meant that it was impractical to carry out all assays in the tubes used during selection. However, growth measured in 96-well plates is strongly correlated with growth measured in tubes (rSB = 0.93, n = 40, p < 0.001, data not shown). The plates were then kept at 28°C for 48 h, the same as between transfers during selection. Cell number was then estimated by measuring optical density at 650 nm using an EMax precision microplate reader (Molecular Devices Corporation). To obtain growth scores, optical densities were corrected by subtracting control well scores (sterile media).

(c) Measurement of diversity

To quantify the overall phenotypic diversity within each evolved population, we used Euclidean distances to measure phenotypic differences between the genotypes (Barrett & Bell 2006). For two genotypes from the same population, the Euclidean distance is the square root of the sum of the squared differences in growth between the two genotypes over all substrates. Biologically, this measures differences in the metabolic profiles of two genotypes. Thus, by taking the mean Euclidean distance over all pairwise combinations of genotypes in a sample, we quantified phenotypic diversity in each population. However, there are different kinds of diversity that can give rise to high Euclidean distances, and since we are interested in niche diversification in particular, we used further analyses to distinguish different types of diversity.

Within-population variation in growth rates was analysed by ANOVA using JMP v. 5.1 software (SAS Institute). Variance was partitioned into genotype, environment and genotype-by-environment interaction components. It is the interaction component that we are particularly interested in, since this reflects environment-dependent differences between genotypes; therefore, it indicates the emergence of different phenotypes adapted to different combinations or ratios of substrates.

Genotype-by-environment interaction was further decomposed into two components, inconsistency and responsiveness, as described by Bell (1990; see also Barrett et al. 2005). Inconsistency variance is due to contrasting correlations among genotypes over environments. If we consider that for each genotype the four carbon substrates can be ranked in terms of growth, then inconsistency indicates variation among genotypes of the order of substrate rankings. For example, in a given population, one genotype may perform best on glycerol while another genotype grows fastest on succinic acid. Thus, high inconsistency suggests that different genotypes within a population are adapted to metabolize different subsets of the
available resources. Responsiveness variance is due to differences in environmental variance among genotypes. For example, if some genotypes within a population grow to a similar extent on all substrates while other genotypes grow better on some substrates than on others, then this would generate responsiveness variance. Thus, genotype-by-environment interaction implies different patterns of adaptation between genotypes, and high inconsistency would support our interpretation of a strong interaction component as an indication of niche diversification.

In fitting models, environment (assay substrate) was taken as fixed and genotype as random. Once different types of diversity had been calculated for replicate populations selected in each of the selection environments, we tested for variation between environments with different resource supply rates by one-way ANOVA. Prior to comparison between selection environments, data for Euclidean distances and environmental variance were log-transformed to account for heterogeneity of variance; figures 1 and 2 show back-transformed data.

3. RESULTS

(a) Response to selection was positive in all populations

Evolved lines from all selection environments showed increased growth relative to the ancestral strain in each assay substrate (figure 1). After approximately 500 generations of selection, this suggests considerable adaptation to substrates within complex environments.

(b) Population density varies with resource supply rate

By manipulating the total concentration of carbon substrates, we enacted variation in resource supply rate between selection environments so that population density increased with resource supply rate ($F_{5,41} = 266.82$, $p < 0.0001$; figure 2a) and saturated at high levels, so that further increases in resource input did not increase population density.

(c) Total phenotypic diversity increases with resource supply rate

Populations selected at higher resource supply rates show greater overall phenotypic diversity, measured as mean Euclidean distance over all pairwise combinations of genotypes ($F_{5,41} = 12.41$, $p < 0.0001$; figure 2b). However, this is an overall measure of metabolic differences between genotypes, and this can indicate either the existence of different types adapted to different niches or simply variation in performance between individuals of the same type. Thus, we took variation in growth owing to genotype-by-environment interaction as a measure of niche differentiation.

(d) Niche diversification peaks at intermediate resource supply rates

Genotype-by-environment interaction is affected by resource supply rate ($F_{5,41} = 7.84$, $p < 0.001$; figure 2c) and peaks at intermediate levels. This shows that in environments with intermediate resource supply rates, different genotypes had adapted to different combinations or ratios of substrates, constituting distinct niches. We further partitioned the interaction variance into inconsistency and responsiveness to clarify whether the different types within each population had adapted to different combinations of substrates, or had adapted to the same substrates in varying degrees. Inconsistency varies between selection environments ($F_{5,41} = 15.36$, $p < 0.0001$) and is greatest at intermediate resource supply rates, suggesting that high genotype-by-environment interaction in these lines was due to the existence of genotypes that had adapted to different combinations of substrates, indicating niche diversification.

We examined the environmental variance component for each population to test for differences in average growth among the different substrates. This was also affected by resource supply rate ($F_{5,41} = 6.50$, $p < 0.0001$; figure 2d), and it is greatest at the highest level, indicating a population specialized to a single substrate. Low environmental variance can suggest either a breadth of
adaption or a mixture of genotypes equally adapted to different substrates. Given the trend for niche diversification and overall diversity, it is clear that low environmental variance at low resource supply rates indicates relatively low diversification and the existence of generalists, while low environmental variance at intermediate resource supply rates is due to equivalent growth of a range of genotypes adapted to different niches.

In summary, at low resource supply rates, niche diversification and overall phenotypic variation are relatively low; at intermediate levels, different genotypes had adapted to distinct niches and diversity is increased; at very high resource supply rates, overall diversity is high but is due to variation within the same phenotypic class that had specialized to a particular substrate.

4. DISCUSSION

In common with previous work (Barrett et al. 2005), we find that initially uniform populations of P. fluorescens can diversify in a complex environment. Different genotypes emerge that have different performance profiles across the available resources. However, results show that the degree of diversification depends critically on the level of resource supply. While overall phenotypic diversity increased with resource supply, our measure of niche differentiation, which can be thought of as analogous to species richness, peaked at intermediate levels. We can relate these findings to the effects of resource supply on competition and ecological opportunity.

One possible explanation for the fact that overall phenotypic diversity increases with resource supply rate is the effect of variation in population size between environments. Since the serial transfer procedure used during selection involved transferring a fixed volume of media each time, larger populations in environments with higher resource supply rates were maintained throughout the period of selection. In larger populations, the number of random mutations per generation is higher (Gerrish & Lenski 1998), potentially leading to the maintenance of higher genetic variance for fitness. The loss of genetic variability by random drift is also generally slower in large populations (Amos & Harwood 1998), but the effective population sizes in all our selection environments were large enough that drift is unlikely to be an important factor in the observed pattern. Since variation in growth among individuals of the same metabolic phenotype can account for high Euclidean distances, high overall phenotypic diversity does not necessarily indicate stable diversifying selection. This applies at very high resource supply rates, where populations comprise a single phenotype that is specialized to a particular substrate.

In contrast to the patterns of overall phenotypic diversity, niche diversification was greatest in environments with intermediate resource supply. How can such a unimodal distribution be explained? When resources are scarce, diversification may be limited owing to a lack of ecological opportunity. Here, selection favours only a generalist phenotype that can use a wide range of the available resources. As resource supply increases, there is a greater number of viable niches (Bell 1997), here meaning different subsets of the available carbon sources. Therefore, the benefits of specialization to a particular niche will increase, provided that the population does not adapt equally well to all available niches simultaneously (Levene 1953). In this case, competition for resources may generate divergent selection, favouring adaptation to underexploited niches and the evolution of genotypes adapted to different niches. At very high levels of resource supply, population growth within a given niche is no longer limited by the availability of resources. At this point, there will be little selection for diversification and...
instead the population adapts to exploit the most abundant or productive resource. For example, if a particular resource is more easily exploited, then the most successful genotypes are those that are best adapted to it and ecological diversity will be low.

Our results are consistent with known principles of bacterial resource use. When presented with a range of substrates at high concentrations, bacteria generally use them sequentially, first metabolizing the most easily exploited and then switching to other substrates as this becomes depleted (Harder & Dijkhuizen 1982). Therefore, if the preferred substrate is maintained at very high levels, as in our high resource input treatments, the population may never switch to exploit alternatives. Here, only the ability to use the preferred substrate will be exposed to direct selection, leading to specialization to this substrate. In contrast, at low concentrations of resources, exploiting a single substrate does not permit significant growth, and hence bacteria typically use multiple substrates simultaneously (Lendenmann et al. 1996). In this case, performance on all substrates is exposed to selection and a generalist strategy may evolve.

Having invoked adaptation to particular substrates or combinations of substrates, we might now ask how adaptation to one substrate affects the ability to exploit alternatives. The stable maintenance of diversity is often predicted to rely upon negative correlations of fitness between alternative habitats or resources (Levene 1953; Futuyma & Moreno 1988; Kawecki 2000). That is, if adaptation to a specific niche does not incur a reduction in fitness elsewhere, then a generalist phenotype would dominate across the environment (Via & Lande 1985). Thus, in the context of bacteria in complex environments, if there is no fitness trade-off between alternative substrates, then we would ask why different phenotypes emerge instead of a generalist that adapts by increasing its performance on all substrates. At first our results appear to show no such cost of adaptation, since growth increased on all substrates. This agrees with the previous findings that when bacteria adapt to particular resources, a cost of adaptation may or may not be sustained (Velicer 1999; Velicer & Lenski 1999; MacLean & Bell 2006). However, diversification can still proceed, provided that the correlated response to selection is weaker than the direct response (Bell 1997). Hence, even though adaptation to a particular substrate may also increase the affinity for alternatives (Clarke 1984; Lin & Wu 1984; Mortlock 1984), a fitness trade-off exists if the increase in growth is greater on one substrate than on the other.

The mechanism by which adaptive diversification proceeded in this experiment is fundamentally different from that described by Kassen et al. (2000, 2004). In static liquid microcosms, diversification results from adaptation to spatially discrete niches, and a unimodal relationship between diversity and resource supply is explained only in the context of spatial heterogeneity. In contrast, we find that selection based solely on exploitation of available resources can explain patterns of diversification and that the outcomes of this process are strongly affected by the availability of resources in the environment. Given broad theoretical evidence that competition for resources and ecological opportunity are key components of divergent evolution (Simpson 1953; Schluter 2000), the mechanism described here may underlie several well-known patterns of diversity.

Increases in resource supply to very high levels frequently lead to a drop in diversity (Rosenzweig 1971; Tilman 1987; Kassen et al. 2000). This ‘paradox of enrichment’ has only been partially explained but is of particular relevance, given the environmental impact of human activities, such as the widespread use of fertilizers (Vitousek et al. 1997; Tilman et al. 2001). We find that diversification does indeed decrease at very high levels of resource supply, despite high population density, this is due to unchecked growth at a single niche resulting in the dominance of a specialist phenotype. The same principle explains the dominance of individual plant species in eutrophicated ecosystems. However, in most natural communities, resources are not constantly available in abundance and the decline in diversity at high resource supply rates is observed only in cases of significant nutrient enrichment.

The relationship between diversity and resource supply has been observed at a range of spatial scales. For example, one of the best-known patterns of diversity is a latitudinal gradient in species richness (Rosenzweig 1995; Gaston 1996; Brown & Lomolino 1998; Rohde 1992). Rising diversity from the poles to the equator appears to reflect changes in energy availability (Wright 1983; Kerr & Packer 1997). Our results suggest that this may be because the limits of diversification are extended when there is a greater resource supply, and therefore greater ecological opportunity. The effect of resource supply may be a general, although not exclusive, explanation for variation in the outcomes of adaptive diversification.

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