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Adaptation Limits Diversification of Experimental Bacterial Populations

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Adaptation to a specific niche theoretically constrains a population's ability to subsequently diversify into other niches. We tested this theory using the bacterium *Pseudomonas fluorescens*, which diversifies into niche specialists when propagated in laboratory microcosms. Numerically dominant genotypes were allowed to diversify in isolation. As predicted, populations increased in fitness through time but showed a greatly decreased ability to diversify. Subsequent experiments demonstrated that niche generalists and reductions in intrinsic evolvability were not responsible for our data. These results show that niche specialization may come with a cost of reduced potential to diversify.

Despite much theoretical (*1–6*) and empirical (*7–14*) work investigating the conditions promoting the evolution of sympatric (within-population) diversity, little is known about the constraints. Here, we demonstrate that adaptation itself is likely to limit a population's ability to diversify, when evolution occurs in "rugged fitness landscapes." Fitness landscapes are created by plotting fitness against all possible genotypes within the population (*4–6*, *15*, *16*). Assuming large populations, selection drives populations uphill toward fitness peaks (adaptation). If several ecological niches exist and organisms show fitness trade-offs between niches (niche specialization), or genetic interactions (epistasis) are a major determinant of fitness, the fitness landscape may be rugged, with multiple peaks and valleys (*4–6*, *15*, *16*). When a population is poorly adapted and in a fitness valley, multiple peaks can be ascended, hence the potential for high rates of diversification. However, as the population ascends a peak, mutations of increasingly large effect, or a number of concurrent mutations of small ef-

fect, will be required for mutants to be competitive when ascending an alternative peak, reducing the potential to diversify.

Consider an environment with two available niches (1 and 2) with the population being regulated at the level of the niche, such that half the population derives from each niche. The fitness of an individual in each niche (W_i) depends on the value of a phenotypic trait (I), which can vary between 0 and 1, and there is a trade-off between fitness in each niche such that $W_1 = f(I)$ and $W_2 = f(1 - I)$. Using Levene's classic model of species coexistence (*1*), we can consider the size of the phenotypic (mutational) change required for a population partially adapted to

niche 1 ($I > 0.5$) to diversify (i.e., for a mutant specializing in niche 2 to be able to invade and be maintained). For a variety of different functional forms of W_i, we find that the better adapted the population to niche 1 (increasing I), the greater the phenotypic effect of a mutation required for invasion of niche 2 (Fig. 1). If the probability of a mutation is inversely proportional to its phenotypic effect, then the specialization will make diversification less likely.

We tested the hypothesis that adaptation within an existing niche in rugged fitness landscapes constrains diversification using the common plant-colonizing bacterium *Pseudomonas fluorescens* (*8*). Rejection of the hypothesis would imply that the landscape is not very rugged relative to the magnitude of mutational effect. When propagated in spatially structured environments (a static glass bottle containing nutrient-rich medium), isogenic *P. fluorescens* populations rapidly diversify, generating numerous niche specialist genotypes that are readily distinguished by their (heritable) colony morphologies on agar plates (*8*). The genotypes can be grouped into three distinct classes on the basis of colony morphology and niche occupation. Smooth (SM) morphs resemble the ancestral genotype and largely inhabit the same, liquid-phase, niche; wrinkly-spreader (WS) morphs form a mat at the air-broth interface, and the much less frequent fuzzy-spreader (FS) morphs colonize the harsher, less aerobic bottom of the vials. Fitness trade-offs between niches have been demonstrated (*8*, *17*) by the

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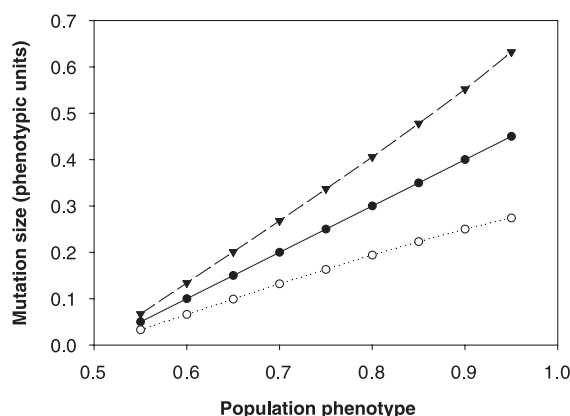*To whom correspondence should be addressed. E-mail: bssagjb@bath.ac.uk

Fig. 1. Magnitude of mutational effect required for adaptation to a new niche, hence diversification, as a function of adaptation to niche 1 (population phenotype). Different lines show different relationships between phenotype (I) and fitness in niche 1 (W_1): $W_1 = I$ (●); $W_1 = I^2$ (▲); $W_1 = I^{0.5}$ (○). Fitness functions in niche 2 (W_2) are the same as in niche 1, but with I replaced with $1 - I$.

operation of negative frequency-dependent selection; genotypes have a fitness advantage when rare because there is less intense competition within their niche (18, 19).

To investigate whether adaptation within existing niches constrained diversifications, we initiated six *P. fluorescens* populations with a single clone [SBW25 (20)] and propagated them for a week in spatially heterogeneous environments. Diversity was determined from colony morphologies on agar plates (8, 10, 17, 21), and the numerically most dominant SM-like genotype in each population was isolated and used to initiate a single subsequent culture, which was propagated as above. This procedure was repeated five times for each of the six replicate lines, resulting in one genotype from each of six transfers for each line (a total of 36 evolved clones) (22). Consistent with the evolved genotypes occupying the SM niche, the six clones from the final transfer were able to competitively exclude ancestral SM (22). In all lines, diversification after six transfers was much less than ancestral diversification, and there was an overall negative correlation between transfer number and ability to diversify (Fig. 2A) (sign test of correlations between number of genotypes and transfer number for the six lines: P = 0.03). Different colony types dominated in different populations, suggesting the ascent of different adaptive peaks. However, individual evolved genotypes (that showed little diversification) produced almost identical

suites of novel genotypes when they were allowed to diversify in replicate (22), suggesting that the reduction in diversification is due, to some extent, to a deterministic loss in accessible genotypes during the climbing of a particular adaptive peak.

Our proposed explanation for this decreasing ability to diversify (with increasing transfer number) is niche-specific adaptation. We tested this hypothesis by competing all genotypes from all time points with a genetically marked isogenic mutant of the ancestral genotype in the selective environment (22). Fitness of genotypes did indeed increase through time in all replicates (Fig. 2B) (paired *t* test of ancestral versus transfer 6 selection rate constant: *t* = 15.27, P < 0.0001; sign test of correlations between fitness and transfer number for the six lines: P = 0.03). Furthermore, there was an overall negative correlation between fitness and ability to diversify (sign test of correlations between fitness and number of genotypes for the six lines: P = 0.03).

It is possible that the inability to diversify is the result of types evolving that are superior in all niches (23). If this were the case, we would expect diversity to decrease (as a result of the evolution of generalists) under conditions in which the selection regime involves propagation of a sample of the whole population. We evolved six populations under identical conditions as above, but transferring 1% of the complete culture to fresh media, rather than individual genotypes. We found no evidence of diversity decreasing through time, refuting generalist evolution as the main explanation for our data (sign test of correlations between number of genotypes and transfer number for the six lines: P > 0.2).

A second explanation is an intrinsic reduction in evolvability (for example, reduced mutation rates) through time, possibly as a result of genetic drift caused by our selection regime. Such intrinsic reductions are likely to be apparent in all environments; hence, the same relationships between time spent evolving and diversification should be apparent in a novel environment. Conversely, if ascending local fitness peaks are responsible for the inability to diversify (our hypothesis), diversification in a novel environment should be independent of adaptation to the selective environment: All genotypes are equally unlikely to be approaching fitness peaks in the fitness landscape created by the novel environment. To examine this hypothesis, we measured the ability of each of the genotypes to diversify in two novel environments: in spatially homogeneous environments (shaken microcosms) and in spatially heterogeneous environments (static microcosms) with low nutrients (22). Diversification of the ancestral genotype was considerably less in both environments than in the original environment

Fig. 2. Diversification and fitness through time. (**A**) The ability of genotypes to diversify and (**B**) their fitness (selection rate constant) against a standard competitor, where a value of zero indicates equal performance of the two competing genotypes, after 1 week of growth in static KB media microcosms, as a function of transfer number. Different lines and symbols represent the six replicate lines.
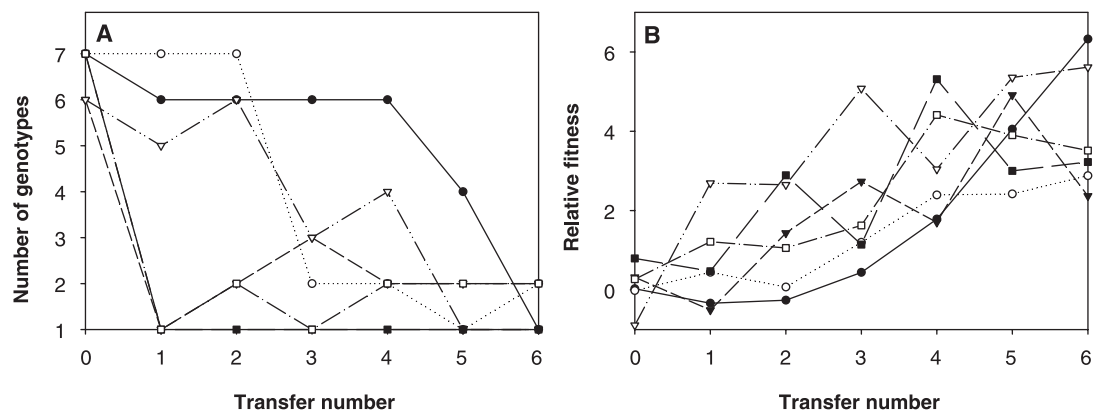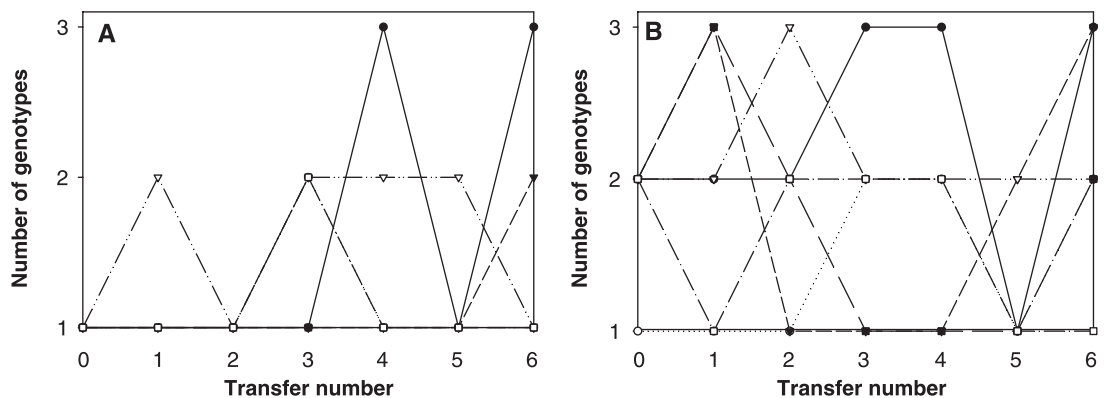

Fig. 3. Diversification in novel environments. The ability of genotypes to diversify in two novel environments: (**A**) shaken KB media microcosms and (**B**) static minimal media microcosms as a function of transfer number. Different lines and symbols represent the six replicate lines.

(compare Figs. 2A and 3). Nevertheless, in both environments there was no relation between ability to diversify and time spent evolving in the original high-nutrient media (Fig. 3) (sign tests of correlations between number of genotypes and transfer number for the six lines: $P > 0.2$ in both cases).

Adaptation can limit the ability of bacterial genotypes to diversify genetically. This was not the result of generalist evolution or the evolution of an intrinsic reduction in evolvability, but was caused by environment-specific adaptation. Given the strong empirical support for both the importance of environmental heterogeneity in diversification (7–14) and epistasis (6, 24), it is likely that rugged fitness landscapes, a requirement for the observed effects, are common. These results are therefore likely to be generally relevant and may help to explain patterns of diversity over both micro- and macroevolutionary time scales. Consistent with recent interpretations of macroevolutionary adaptive radiations (25), we predict that in environments that can potentially support similar levels of diversity, diversification is more likely to occur immediately following colonization of the environment than through expansion into new niches within the environment after an extinction event.

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Melt Inclusions in Veins: Linking Magmas and Porphyry Cu Deposits

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At a porphyry copper-gold deposit in Bajo de la Alumbrera, Argentina, silicate-melt inclusions coexist with hypersaline liquid- and vapor-rich inclusions in the earliest magmatic-hydrothermal quartz veins. Copper concentrations of the hypersaline liquid and vapor inclusions reached maxima of 10.0 weight % (wt %) and 4.5 wt %, respectively. These unusually copper-rich inclusions are considered to be the most primitive ore fluid found thus far. Their preservation with coexisting melt allows for the direct quantification of important ore-forming processes, including determination of bulk partition coefficients of metals from magma into ore-forming magmatic volatile phases.

In porphyry ore deposits, metals are concentrated by large volumes of magmatic volatiles exsolved from crystallizing upper crustal magma bodies (1). Whether an ore deposit forms depends on the availability of metals in the magma, the partitioning of those metals into the volatile phase, and the history of the fluid after release from the magma (2–4). Ores are typically associated with hydrothermal mineral assemblages produced by the interaction of magmatic fluids with wall rocks (5). The earliest formed hydrothermal alteration of wall rocks is potassic (biotite-K-feldspar-quartz with or without magnetite assemblage) and is caused by high temperature (350° to 800°C) and saline (up to 70 weight % equivalent NaCl) fluids (6, 7). Discovery of silicate-melt inclusions coexisting with fluid inclusions in magmatic-hydrothermal quartz veins unambiguously links devolatilization of the magma with the associated porphyry ore deposit. This occurrence preserves the most primitive magmatic volatiles and the melt from which these were derived and, with the use of advanced microanalysis techniques, allows chemical changes to be traced through the evolution of the hydrothermal system. Moreover, we are able to use those data to quantify the magmatic-hydrothermal processes that lead to the formation of porphyry Cu deposits.

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Bajo de la Alumbrera is an Au-rich porphyry Cu deposit where potassic alteration assemblages overprint several phases of porphyritic dacite intrusions and are associated with the bulk of the disseminated Cu-Fe sulfides and Au. High temperature (maximum of 750°C) and saline fluid (>35 wt % equivalent NaCl) of magmatic origin (as inferred from the calculated $\delta^{18}O$ and δD compositions) formed these alteration assemblages (8). Some of the earliest Cu-Fe sulfides occur in diffuse quartz veins, which are texturally similar to those described as A veins (9); however, the presence of silicate-melt inclusions warrants a new vein subclass. Hereafter we refer to them as P veins, reflecting their primitive role in the evolution of the magmatic-hydrothermal system. Typically, they consist of sugary quartz, with lesser amounts of K-feldspar and with or without hornblende-biotite-magnetite-chalcopyrite ($CuFeS_2$) and pyrite.

The P veins contain silicate-melt inclusions that consist of silicate crystals, vapor bubbles, salt crystals, and opaque oxide and sulfide crystals (Fig. 1). These inclusions are similar to those in magmatic quartz phenocrysts in the mineralized intrusions (10). Heating experiments (11) on silicate-melt inclusions revealed consistent phase transformations: The dissolution of the salt phases (e.g., halite and sylvite) occurs between 105° and 560°C; dissolution of crystalline silicate phases occurs between 650° and 765°C. After heating the host quartz crystals to 800°C at 1 atm external pressure for several hours, the sample was quenched to produce a silicate glass, which is extremely rich in K_2O compared to whole rock analyses and has a composition similar to K-feldspar (table S2). This composition may represent a chemically modified melt trapped in a dominantly aqueous environment or may contain ex-