Adaptive radiation in a heterogeneous environment

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Adaptive radiation in a heterogeneous environment

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Successive adaptive radiations have played a pivotal role in the evolution of biological diversity[1–3]. The effects of adaptive radiation are often seen[4–6], but the underlying causes are difficult to disentangle and remain unclear[7–9]. Here we examine directly the role of ecological opportunity and competition in driving genetic diversification. We use the common aerobic bacterium *Pseudomonas fluorescens*[10], which evolves rapidly under novel environmental conditions to generate a large repertoire of mutants[11–13]. When provided with ecological opportunity (afforded by spatial structure), identical populations diversify morphologically, but when ecological opportunity is restricted there is no such divergence. In spatially structured environments, the evolution of variant morphs follows a predictable sequence and we show that competition among the newly evolved niche-specialists maintains this variation. These results demonstrate that the elementary processes of mutation and selection alone are suifficient to promote rapid proliferation of new designs and support the theory that trade-offs in competitive ability drive adaptive radiation[14,15].

Explanation of macroevolutionary phenomena (for example, adaptive radiation and punctuated evolution) by direct extrapolation from microevolutionary processes (for example, mutation and competition) is contentious[1,16–18]. Conventional explanations for adaptive radiation frequently invoke no more than vacant niches and stringent competition between niche specialists[14,19–21]. Experimental studies have lent credence to this view[22], but by necessity

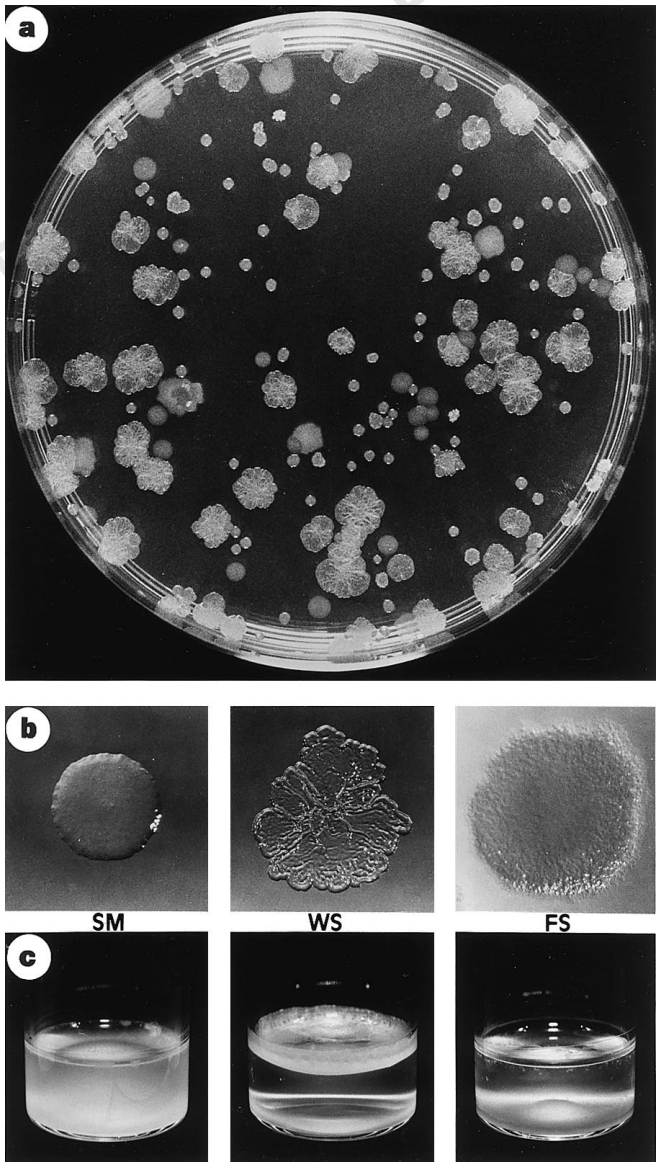

Figure 1 Phenotypic diversity and niche specificity among *P. fluorescens* SBW25 colonies evolved in a spatially heterogeneous environment. Populations were founded from single ancestral 'smooth' (SM morph) cells and propagated in 6-ml King's medium B contained in a 25-ml microcosm at 28 °C. Microcosms were incubated without shaking to produce a spatially heterogeneous environment. **a**, After 7 days, populations show substantial phenotypic diversity which is seen after plating. **b**, Most phenotypic variants can be assigned to one of three principle morph classes: (SM), wrinkly spreader (WS) and fuzzy spreader (FS). **c**, Evolved morphs showed marked niche preferences.

these studies have been limited in scope[7,15,21]. An ideal experiment would follow the evolution of a single genotype in multiple environments that differ solely in their niche potential. The experiment would be designed such that ecological and genetic factors could be carefully controlled, thus allowing mechanistic hypotheses concerning the origin and maintenance of diversity to be tested.

Bacteria are well suited for such a study. They are easily propagated, have rapid generation times and large populations sizes, and can be stored in a state of suspended animation so that the fitness assays of evolved and ancestral genotypes can be performed by direct competition[9,23]. As bacteria reproduce asexually, identical populations can be established from a single genotype; all subsequent variation is therefore generated *de novo*, by mutation. *P. fluorescens* (SBW25) populations evolve rapidly in novel environments[12]. Evolution is seen at the phenotypic level in differences in colony morphology, which are easy to detect (Fig. 1), heritable and genetically based (see Methods). A striking feature of the evolved morphs is their niche specificity (Fig. 1c). The fortuitous relationship between colony morphology and niche preference allows real-time ecological and evolutionary dynamics to be determined by scoring changes in the frequencies of colony phenotypes.

To investigate the effect of ecological opportunity on the evolution of genetic diversity, we propagated replicate isogenic populations of the ancestral (smooth) morph in spatially heterogeneous environments provided by static broth cultures (microcosms; Fig. 1). Microcosms were destructively sampled every 24 h and the evolution of diversity was determined by monitoring changes in the frequencies of colony morphologies. After 3 days, extensive morphological diversification was evident and this was sustained over 10 days (Fig. 2a). The three dominant morphs (named smooth, wrinkly spreader and fuzzy spreader) showed highly repeatable evolutionary dynamics across populations (Fig. 2c). This fact and the rapid rise in diversity indicated that strong, diversifying selection was occurring. To test whether ecological opportunity, afforded by multiple vacant niches, was a crucial factor in the evolution of diversity, we propagated replicate populations of the ancestral (smooth) morph as before, but destroyed the physical structure of the environment by shaking the cultures, thereby eliminating the spatial heterogeneity and the multiplicity of niches. In this essentially homogeneous environment, there was no morphological variation (Fig. 2b, d).

Initial diversification is not necessarily indicative of sustained diversity[4,19] and it is possible that the differences in morphological diversity between populations in the two environmental regimes were transient. Variation in the heterogeneous environment may have resulted from sequential sweeps of adaptive mutants that would eventually leave a single dominant phenotype[24]. Alternatively, successive selective sweeps of undetected traits may have prevented morphological diversification in the homogeneous environment. To investigate these possibilities, we propagated a set of ten isogenic ancestral (smooth) populations under the spatially heterogeneous regime. After 7 days these cultures, as expected, became genetically diverse. We then determined whether the maintenance of diversity depended on continued environmental heterogeneity. We used a sample from each of the ten genetically diverse populations to found two new populations, one set of which was evolved for two successive 7-day periods under spatially heterogeneous conditions, the other of which was evolved under spatially homogeneous conditions (Fig. 3). Populations in the heterogeneous environment showed sustained levels of high diversity, whereas populations switched to the homogeneous environment suffered a rapid loss of diversity. These results indicate that selection is important in the maintenance of morphological diversity in the heterogeneous environment, but leads to purging of variation in the homogeneous environment.

If selection is the primary evolutionary force maintaining diversity in the heterogeneous environment, then competitive trade-offs among different niche-adapted genotypes would be expected[14,15,21,25]. Such trade-offs are likely to be frequency-dependent[26]: that is, genotypes will have a fitness advantage when they are rare that disappears when they are common. To assess this, we examined the ability of the three principal morphological types to invade, when rare, a population of a different morph type. The

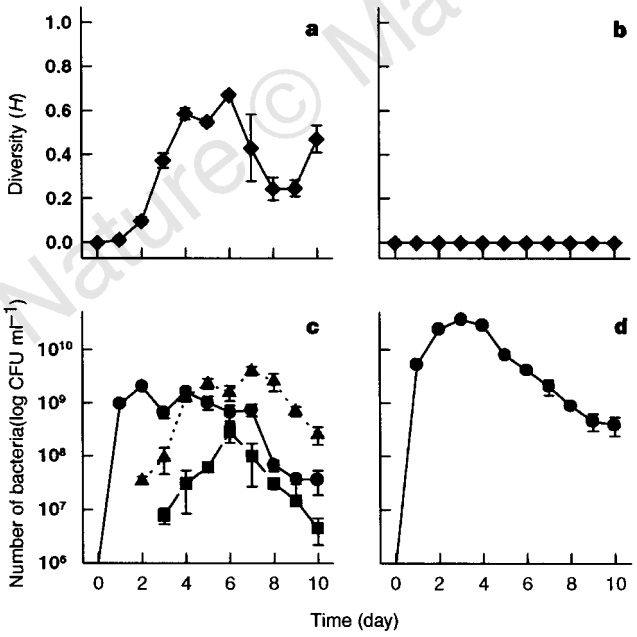

Figure 2 Effect of ecological opportunity on the evolution of genetic diversity. Two sets of identical populations were founded from the ancestral smooth morph and incubated either with or without shaking. Every 24 h, three replicate microcosms were destructively collected and diversity was determined by scoring the frequencies of morphs. **a**, **b**, Diversity increased rapidly in the heterogeneous environment (**a**) whereas no diversity was detected in environments lacking spatial structure (**b**). **c**, **d**, Evolutionary dynamics of the principal morph classes in the heterogeneous (**c**) and homogeneous (**d**) environments. Values are the means ± s.e.m. ($n = 3$). Circles represent smooth morphs, triangles represent wrinkly-spreader morphs and squares represent fuzzy-spreader morphs.

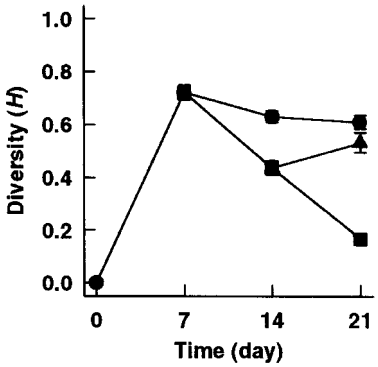

Figure 3 Effect of spatial heterogeneity on the maintenance of genetic variation. Ten replicate populations founded from the ancestral smooth morph were allowed to evolve in static microcosms. After 7 days, a sample of ~4×10^4 cells from each microcosm was used to found two fresh sets of ten replicate microcosms; one set was incubated under spatially homogeneous conditions (squares) and the other under spatially heterogeneous conditions (circles; upper line). These populations were propagated for two additional 7-day periods. Diversity in homogeneous microcosms declined significantly relative to their heterogeneous partners ($P < 0.001$, one-tailed test). Propagation of day-14 homogeneous microcosms under heterogeneous conditions restored diversity (triangle). Values are the mean ± s.e.m. ($n = 10$).

single ancestral (smooth) genotype and 24 independently derived isolates from each of the wrinkly-spreader and fuzzy-spreader evolved classes were competed in a pairwise manner. The ratio of competing genotypes at the beginning of each experiment was ~100:1, and populations were competed for 7 days to determine the fitness of the initially rare genotype relative to the common genotype by scoring frequencies on agar plates. Use of a neutral pantothenate marker allowed us to distinguish between invasion of the initially rare genotype and phenotypically visible diversification of the common genotype. From three sets of pairwise competitions (smooth versus wrinkly spreader, smooth versus fuzzy spreader and fuzzy spreader versus wrinkly spreader), two interactions resulted in stable maintenance of variation (Fig. 4), namely the interactions involving the ancestor and derived morphs (smooth versus fuzzy spreader or wrinkly spreader). The detection of competitive trade-offs between niche specialists (frequency-dependent selection) confirms predictions of population genetics theory and indicates that the engine of adaptive radiation was competition[14,25].

The ecological mechanisms maintaining diversity within the spatially heterogeneous environment are complex and not fully understood. However, competition for oxygen seems to be a significant factor. Cells of the wrinkly-spreader morph adhere firmly to each other and to surfaces, allowing the formation of a self-supporting mat (Fig. 1). Occupation of the air–broth interface provides WS cells with access to both oxygen and nutrients and so they are selectively favoured at the expense of genotypes within the oxygen-poor broth phase. Once the wrinkly-spreader morph becomes common, its fitness declines because the weight of the mat increases beyond the point at which it is self-supporting, and it sinks. The selective advantage of the fuzzy-spreader morph is unclear, but its persistence appears to depend on the stable frequency-dependent selection between the smooth and wrinkly-spreader morphs.

It is striking that complex ecological interactions within the spatially heterogeneous environment evolved over such a short period of time, and that the interactions were nearly identical across microcosms. The rapidity and repeatability of evolution here are indicative of both strong selective pressures and the adaptive potential of the ancestral genotype, despite the underlying random nature in the appearance of mutations. However, the apparent determinism of the system is not all-encompassing. First, although we detected three dominant morphological classes in every microcosm, variation was always encountered within the morphological classes across microcosms, and different, minor morphological classes appeared (we have not described these here). Second, in experiments performed in spatial microcosms one-hundredth the size of those described above, diversification resulted in far less predictable patterns, indicating that the apparent determinism is due, in part, to large population sizes and hence large numbers of mutants. Finally, persistence of the three dominant morphological classes indicates that multiple avenues for adaptation existed for the ancestral genotype, and shows that different cell lineages followed different adaptive pathways.

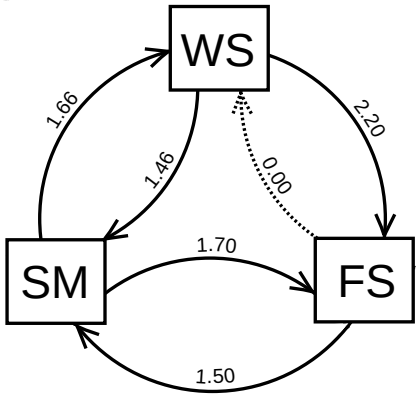

Figure 4 Competitive relationships among niche-adapted classes. Arrows point from the initially rare (invading) morph to the common (invaded) morph for each of the competitions. Values above each curve are the median fitnesses. Fitness measures were determined over 7 days by the ratio of Malthusian parameters of the initially rare genotype to the common genotype, so that a fitness of 1.00 indicates genotypes of equal competitive ability[23]. In five out of six cases, the rare morph successfully invaded the common morph, whereas in one case (FS invading WS) the rare genotype was selected against. All interactions are statistically significant ($P < 0.01$) by two-tailed tests.

Variation is extensive within populations of many organisms and is thought to correlate with environmental heterogeneity[27]. In bacteria, variation within a clonal lineage is commonplace[11–13]; stable polymorphisms have been observed in experimental populations[28,29] and variation in pathogen populations is evident in plate culture—in some instances the molecular basis for the variation is known[12,13]. Despite intensive study, the causes and significance of this variation have remained elusive[11–13,30]. By showing that different variants occupy different niches and that competition between variants drives diversification, we reveal both the ecological causes and the evolutionary significance of morph variation within *P. fluorescens* populations. In so doing, we provide a framework within which to consider the significance of phenotypic variation in other bacteria.

Rigorous tests of the significance of ecological opportunity in adaptive radiation have been limited[1]. Here, within a matter of days we have seen an adaptive radiation that has the hallmarks of macroevolutionary dynamics, including rapid evolution and niche specialization. By examining the evolution of genetically uniform populations in identical environments that differ only in their niche potential, we have shown that spatial heterogeneity is a significant factor affecting not only the occurrence, but also the maintenance, of diversity. Multiple vacant niches provided opportunities for niche specialization and led to the partitioning of genotypes according to habitat. The driving force for this radiation was competition. As populations were founded by a single asexually reproducing genotype, we can attribute the evolution and proliferation of new designs directly to mutation and natural selection. □

Methods

Bacteria. *P. fluorescens* isolate SBW25 was isolated from a sugar beet leaf[10] and propagated in King's medium B (KB). Long-term storage was at −80 °C. A pantothenate auxotroph of *P. fluorescens* SBW25 was generated by deletion of the entire *panB* gene from the ancestral smooth genotype. The marker itself had no discernible effect on fitness in the environments used in this study.

Genetic basis of morph phenotype. Twenty replicate microcosms founded by the pantothenate-marked ancestral genotype ($10\,Pan^+$, $10\,Pan^-$) were propagated for 3 days in static microcosms. A single wrinkly-spreader morph was isolated from each microcosm and grown to stationary phase. Pairs of morphs with opposing markers were mixed and plated onto KB. The correspondence between phenotype and pantothenate marker was determined by growth assays on media supplemented with and without pantothenate. Correspondence was statistically significant ($P = 5 \times 10^{-35}$).

Diversity. Diversity (H) was calculated using the Shannon–Weaver index [$H = (N \log N - \Sigma n \log n_i)/N$], where N is the total number of individuals and n_i is the number of individuals of each phenotype. The phenotypes of at least 100 randomly selected colonies were scored from each microcosm.

Competition experiments. Forty-eight replicate microcosms founded by the smooth morph ($24\,Pan^+$, $24\,Pan^-$) were propagated for 3 and 7 days, after which time 24 wrinkly-spreader and 24 fuzzy-spreader colonies were isolated, respectively. For each of the six interactions between niche-adapted classes, each derived niche-adapted genotype was competed against the common ancestor or a randomly chosen niche-adapted genotype of the other class. We used two-tailed sign tests to assess the statistical significance of the competitions, and all were significant at $P < 0.01$, after sequential Bonferroni correction for carrying out six simultaneous tests.

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Temporal gating of neural signals during performance of a visual discrimination task

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The flow of neural signals within the cerebral cortex must be subject to multiple controls as behaviour unfolds in time. In a visual discrimination task that includes a delay period, the transmission of sensory signals to circuitry that mediates memory, decision-making and motor-planning must be governed closely by 'filtering' or 'gating' mechanisms so that extraneous events occurring before, during or after presentation of the critical visual stimulus have little or no effect on the subject's behavioural responses. Here we study one such mechanism physiologically by applying electrical microstimulation[1–3] to columns of directionally selective neurons in the middle temporal visual area[4–9] at varying times during single trials of a direction-discrimination task. The behavioural effects of microstimulation varied strikingly according to the timing of delivery within the trial, indicating that signals produced by microstimulation may be subject to active 'gating'. Our results show several important features of this gating process: first, signal flow is modulated upwards on onset of the visual stimulus and downwards, typically with a slower time course, after stimulus offset; second, gating efficacy can be modified by behavioural training; and third, gating is implemented primarily downstream of the middle temporal visual area.

Several lines of evidence indicate a critical role for MT (the middle temporal visual area) in the analysis of visual motion information. Roughly 90% of MT neurons respond selectively to stimulus motion within a restricted range of directions[7]. Furthermore, MT neurons are organized in columns such that neighbouring neurons preferentially respond to similar directions of motion[9]. Electrical microstimulation of these columns can bias a monkey's judgements of motion direction towards the direction encoded by the stimulated neurons, showing that directional signals in MT are important in generating psychophysical performance[1–3]. In earlier microstimulation experiments, trains of stimulating pulses were delivered at the same time as presentation of the motion stimulus to

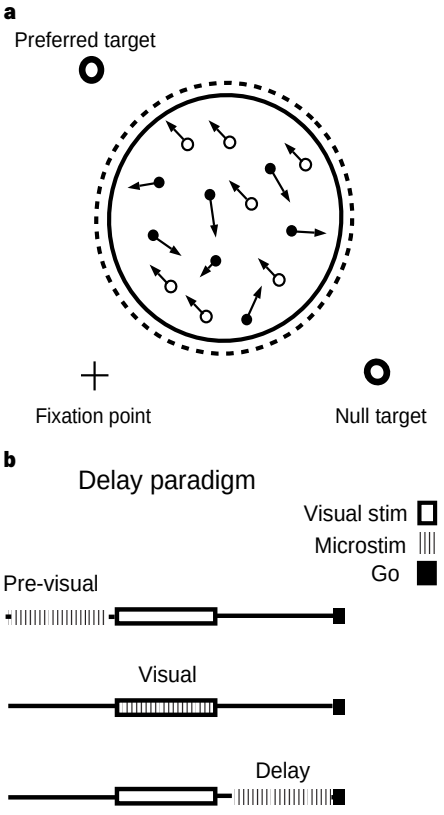

Figure 1 The protocol. **a**, Diagram of the visual display. The visual stimulus aperture (complete circle) was positioned within the multi-unit receptive field (dashed circle) mapped at the microstimulation site. Open dots represent the coherent motion signal (arrows directed upwards and left); solid dots represent the masking motion noise (randomly directed arrows). **b**, The sequence of events. Stimulating pulses were delivered for equal amounts of time in each experimental condition. Control trials with no microstimulation were randomly interleaved among the test trials.