

Microbial experiments on adaptive landscapes

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Summary

The adaptive landscape is one of the most widely used metaphors in evolutionary biology. It is created by plotting fitness against phenotypes or genotypes in a given environment. The shape of the landscape is crucial in predicting the outcome of evolution: whether evolution will result in populations reaching predictable end points, or whether multiple evolutionary outcomes are more likely. In a more applied sense, the landscape will determine whether organisms will evolve to lose 'costly' resistance to antibiotics, herbicides or pesticides when the use of the control agent is stopped. Laboratory populations of microbes allow evolution to be observed in real time and, as such, provide key insights into the topology of adaptive landscapes. *BioEssays* 27:1167–1173, 2005.

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Introduction

The fitness, or adaptive, landscape is one of the most intuitively appealing metaphors in evolutionary biology. The landscape is generated by plotting fitness against genotypes or phenotypes in a given environment.^(1–6) Because natural selection drives populations towards fitness peaks, the shape of the landscape will play a major role in the evolutionary process. A key feature of the landscape, and the focus of this article, is whether there is a single fitness peak (a 'smooth' landscape) or multiple fitness peaks (a 'rugged' landscape). If there exists a single peak, different evolving populations will ultimately converge on the same evolutionary solution to the problems posed by adapting to the environment. By contrast, rugged landscapes will greatly increase the chance of populations finding different evolutionary solutions, and will reduce the probability of populations evolving in reverse, when selection pressures change. This latter point has major implications for medicine and agriculture: will populations that have evolved resistance to antimicrobials, pesticides and herbicides revert to sensitivity when the selective agent is removed?

Unfortunately, obtaining information about the topologies of fitness landscapes is difficult. Many different approaches have been used (reviewed in Refs 4–6), but these have generally been limited to trying to infer the process of evolution from its end points. Here we discuss a different approach to the problem: microbial experimental evolution. Using organisms with a short generation time such as bacteria or viruses, it is possible to follow adaptation in real time in the laboratory under controlled environmental conditions.^(7,8) Microbes can be stored in suspended animation, allowing direct comparisons between evolved and ancestral states.

The main aim of this article is to demonstrate what microbial evolution experiments can tell us about the topologies of adaptive landscapes; this is not intended to be a comprehensive review of experimental evolution studies (see⁽⁹⁾). However, we would also like to emphasise that microbes are not just useful as model systems for understanding evolution in other organisms. Lessons learned about the topology of adaptive landscapes in microbes can have direct relevance to understanding the evolution of medically important microbes around us.

The topology of adaptive landscapes

For illustrative purposes, here we focus on the conceptually simplest landscape manifestation, the individual genotypic (fitness) landscape. To produce such a landscape we begin with a genotype space, which is simply all the genotypes that are possible in our system. Thus, imagine an organism so simple that it has only two genes that affect its fitness (A and B) and that each gene has only two alternative alleles (A or a and B or b). Its genotype space will consist of the four possible genotypes (AB, Ab, aB, ab), and its fitness landscape will be the distribution of fitness over this genotype space. However, even in a simple system like this, we can get different types of landscape (Fig. 1). At one extreme, if the genes affect fitness independently, so that the allele that is best to have at gene A is independent of the allele present at gene B and vice versa (Fig. 1a), the landscape will have a single fitness peak, and can be described as a smooth landscape. However, not all landscapes are smooth. If the best allele to have at each locus changes depending on the allele present at the other locus, the landscape will have multiple adaptive peaks, potentially of different heights (Fig. 1b) connected to each other by lower fitness intermediates. Such a landscape is

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appear. By contrast, if landscapes are smooth, evolution will be more of a deterministic walk to the single optimum genotype. In other words, do initially identical populations evolve in parallel, and do initially different populations converge on the same evolutionary solution?

Experiments with microbes have allowed this question to be addressed directly. Initially genetically identical populations can be allowed to adapt to defined experimental conditions time and time again allowing the repeatability of the evolutionary process to be examined and dissected. One such study using the bacteria *Escherichia coli* has been running since 1988.^(7,12–16) Twelve genetically identical populations were set up in glucose-limited media and have been propagated daily by diluting each population 100-fold. To date, the experiment is in excess of 40,000 generations. All populations showed a relatively rapid increase in fitness (based on competition with the ancestor), but this rate had slowed down dramatically by generation 1000. This slowing of the rate of increase in fitness suggests populations were approaching their fitness peaks. Despite patterns of evolution being very similar between replicate populations, small but significant variation in fitness (and larger variation in other traits, such as cell size) continues to be maintained between these populations. This suggests that they may have reached different fitness peaks, implying a rugged adaptive landscape. Shorter experiments with another bacteria, *Alcaligenes eutrophus*,⁽¹⁷⁾ produced qualitatively similar results.

However, the above studies provide only fairly crude tools for determining the extent to which the populations are following different evolutionary trajectories at the genetic level since potentially very similar phenotypes could be achieved in dramatically different ways. The observation that the populations showed much greater variation in fitness when grown in novel environments provided the first suggestion that the genetic basis for their adaptation may be very different despite their similar fitness in the selection environment.⁽¹⁴⁾ This has since been supported by detailed genetic analysis that has shown that despite changes in expression patterns from the ancestor occurring in the same direction in the same 59 genes of two studied population, mutations in one gene, *spoT*, which was found to account for a significant amount of these changes in expression, only occurred in 8 out of 12 populations, and the location of the mutation differed in all cases.⁽¹⁶⁾ Thus, very similar expression patterns, and thus fitness, could be generated by mutations in both different sites within a gene, and in different genes. Similar evidence for both parallel and divergent genetic changes have been observed in experimental populations of *Ralstonia* spp.⁽¹⁸⁾

The ruggedness of adaptive landscapes implied by these results suggests that not only will chance events during the adaptive process be important in determining where evolution takes the population, but that the initial genetic composition (and hence evolutionary history) of the population will have a

strong effect on how it evolves in the future. A dramatic illustration of this effect is seen in experiments with the RNA bacteriophage Phi6.⁽¹⁹⁾ When genetically different populations of this virus with similar fitness were allowed to evolve, populations initiated with one genotype showed a rapid increase in fitness, whilst populations from a second genotype actually showed a slight drop in fitness. The authors suggest that the populations are on different adaptive peaks, of very different heights, and that, whilst the first population rapidly evolves to the top of its peak, the second begins at the top of its peak and has nowhere to evolve (the drop in fitness is due to a constant input of deleterious mutations). It has become stuck in an evolutionary dead end.

However, work with *E. coli* suggests that such contingency is not always the rule. When the adaptation of populations starting either from the same genotype or from different genotypes was directly compared, the genetic starting point had little effect on the adaptive process. In both cases, the replicate populations seemed to be following slightly divergent evolutionary trajectories, but the amount of divergence in their fitness did not depend on their starting point.⁽¹³⁾

Taken together, the results lead us to conclude that microbial adaptive landscapes are rugged enough to divert the course of evolution. This conclusion is supported by the large amounts of epistasis between mutations in *E. coli*^(20,21) and in an RNA virus:⁽²²⁾ the fitness effects of mutations are contingent on other mutations within the same genome. Despite this, limited difference in the fitness attained suggests that these peaks are of similar height. Thus populations appear to be evolving on rugged adaptive landscapes, in the sense that there are different optimal solutions, but there is little evidence⁽¹⁹⁾ that populations frequently become trapped on local peaks of much reduced fitness.

Evolution in reverse?

The above studies suggest that fitness landscapes even in simple laboratory environments are rugged enough to affect the path of evolution. However, whether the populations have reached truly isolated adaptive peaks or are simply approaching the same peak along different paths, hence at different rates, is a matter of debate. Maybe after 80000 or 100000 generations, the *E. coli* populations would eventually converge on the same genetic solution? Another approach to examine the topology of adaptive landscapes is to ask whether evolution is typically reversible.⁽²³⁾ Suppose we begin with a population that is at a fitness peak, and we then force the population away from the peak. If the landscape is single-peaked, the population will evolve back to where it started but, if the landscape is rugged, the population may find itself on the slopes of a different peak and evolve in a very different way.

Some of the best evidence that evolution is often not reversible comes from studies of antibiotic resistance in

bacteria.^(24–26) Whilst these studies differ in detail, the underlying procedure is similar. Researchers start with a bacterial strain that is sensitive to an antibiotic and presumed to be approaching an adaptive peak in an antibiotic-free environment. The population is then pushed away from this peak either by moving into an environment that contains antibiotic and allowing resistance to evolve, or by directly inserting the antibiotic resistance gene into the original bacteria strain. However derived, the resistant bacteria typically have reduced fitness in the original environment, as would be expected if they had been pushed from their original peak. The question is: if the bacteria are then allowed to evolve in the original antibiotic-free environment, do they return to the original adaptive peak? The answer is generally no. Rather than simply losing resistance by back mutation, the populations adapt via mutations at other sites, which reduce the cost of the resistance (compensatory mutations), but maintain resistance itself.^(24–26) Direct evidence that these populations are climbing different fitness peaks comes from the fact that these compensatory mutations are only selectively beneficial in the resistant background and significantly deleterious in the original sensitive background.⁽²⁴⁾

Conventional wisdom regarding the problem of rising antibiotic resistance in pathogenic bacteria maintained the hopeful outlook that, since resistance carries a cost in antibiotic-free environments, antibiotic resistance would soon be lost if antibiotic use was cut. The rugged nature of the fitness landscape revealed by these studies suggests a far more pessimistic outlook: once resistance has evolved it may be very difficult for it to be lost again. Just how pessimistic our view will become will depend on the generality of these results.

Such irreversibility is unfortunately not just limited to drug-resistance genes, or even to bacteria. Virulent bacteriophage, viruses of bacteria that require cell lysis to release progeny virions, have been employed to treat bacterial infections since the 1920s.⁽²⁷⁾ However, the rise of antibiotic resistance has resulted in renewed interest in recent years. As with antibiotics, bacteria evolve resistance to bacteriophage, and this resistance is frequently costly.⁽²⁸⁾ Unfortunately, removal of bacteriophage does not generally result in loss of resistance, but rather genetic compensation.⁽²⁸⁾ When the RNA bacteriophage phi6 is maintained for some time at very low population size, it accumulates deleterious mutations through genetic drift, and its fitness drops as it is knocked from its adaptive peak. However, when populations are restored to their normal conditions, fitness is rapidly regained, but frequently this is via compensatory changes to other genes, and not via back mutation.⁽²⁹⁾ Note, however, that reverse evolution to the ancestral state could occur with large population sizes, demonstrating that adaptive landscapes become less rugged and easier to traverse when there are larger population sizes and hence greater genetic variation.⁽⁶⁾

Environmental complexity and landscape topology

The fitness value of particular traits will depend on the current environment; antibiotic resistance genes will be beneficial in an environment with high levels of antibiotic but potentially costly in others. Thus, the shape of the adaptive landscape and the position of adaptive peaks will change in different environments. However, what is less clear is whether particular types of environment lead to more rugged adaptive landscapes. It is an intuitively appealing notion that more-complex environments are also more likely to have more-rugged adaptive landscapes. If a single environment contains multiple potential niches and traits that give an organism high fitness in one niche necessarily reduce fitness in other niches,⁽³⁰⁾ this may lead to the kinds of interactions between different genes that cause adaptive landscapes to be rugged. Specifically, alleles that in combination give high fitness in a particular niche, may have extremely low fitness when mixed with alleles that are beneficial in a different niche, leading to an extremely rugged landscape with deep fitness valleys separating high-fitness allele combinations. If this is the case, then environmental heterogeneity may have a key role to play in diversification and adaptive radiation.^(31,32)

Certainly, there is some evidence that more complex environments may generate more rugged landscapes. If replicate populations of *Alcaligenes eutrophus* are allowed to evolve in a structured environment (Petri dish), they show much greater divergence in adaptive paths than populations selected in an unstructured environment (shaken liquid media).⁽³³⁾ Whilst it is impossible to say whether this variation would be maintained in the longer term (the study lasted 1000 generations), the implication is that these populations are evolving on a more-rugged landscape.

However, probably the most-striking and best-studied example of the potential for heterogeneous environments to generate landscapes with multiple peaks comes from experiments in another bacteria *Pseudomonas fluorescens*.^(34–40) Initially isogenic populations of this bacterium propagated in spatially heterogeneous environments (static tube) rapidly diversify into a number of distinct types, which differ genetically and in their colony morphology. Each type is specialised for a particular microenvironment within the test tube: one type forms a mat on the surface of the media where oxygen levels are highest, another resides in the broth, while another occupies the oxygen-depleted conditions at the bottom of the tube. Thus, the bacteria appear to be evolving on an adaptive landscape with at least three distinct peaks corresponding to the different niches within the tube. Different lineages adapt to conditions within the tube by ascending different peaks. The importance of environmental heterogeneity in this diversification can be shown by repeating the experiment but removing the spatial heterogeneity by constantly shaking the tube. Under these conditions, no such diversification is seen,

presumably because the landscape has become much smoother.⁽³⁴⁾

The diversification seen in *P. fluorescens* is very different from that seen in the *E. coli* experiments discussed above in that it is not occurring between replicate populations in different tubes following different evolutionary trajectories on the landscape. Instead, the diversification occurs within the same tube, with different lineages following different evolutionary trajectories within the same population. Indeed, the same pattern of diversification is seen repeatedly in replicate populations. Why doesn't the type that has climbed the highest peak replace all others within a single tube? The reason appears to be that not only is the landscape rugged, but the heights of the peaks are also constantly changing. Imagine a landscape like Fig. 1b, with some of the population at one peak, and some at the other. If the landscape is fixed, then we expect the type occupying the highest peak to increase in frequency due to their higher fitness and to eventually replace the other type. However, suppose the height of the adaptive peak changes as the population evolves, specifically, the height of the peak gets smaller as the proportion of that type in the population on that peak increases, whilst the height of the other peak increases (negative frequency-dependent selection^(10,11)). At some point, the two peaks will come to have the same height and, since both types have the same fitness, equilibrium will be reached. If anything changes, the frequencies of the two types, the heights of the peaks will change to move the population back to the equilibrium. But what would cause the peaks to change like this? It seems that, in *P. fluorescens*, the movement of the landscape is due to competition within the niche. If only a small proportion of the population are using the niche, then there will be little competition, and the fitness of the individuals will be high. However, as more of the population specialises in the niche, competition will increase, and the fitness of each individual will be reduced.

Interestingly, *P. fluorescens* does not always show such rapid diversification, even in a heterogeneous environment. If populations of *P. fluorescens* are allowed to diversify, and then the most numerically dominant genotype that has a 'smooth' morphotype (adapted to the broth phase of the tube) from each population is allowed to diversify again, and this process is repeated several times, two things happen. The fitness of the bacteria increases, but this increase in fitness is coupled with a reduction in diversification. This was presumably because it was less likely for populations increasingly drawing near to their adaptive peak to obtain a mutation (or simultaneous mutations) of sufficiently large effect to start to ascend a new adaptive peak. After six rounds of selection, most clones failed to show any diversification. However, the generality of these results must be treated with caution: populations were exposed to clonal bottlenecks at each cycle, and this reduction in genetic variation is likely to increase the probability of populations getting stuck on specific adaptive peaks because

the lack of genetic variation makes the adaptive landscape more rugged.

Given that replicate populations appeared to follow divergent trajectories, even in simple uniform environments, and that heterogeneous environments appear to generate more-rugged adaptive landscapes, does evolution in replicate complex environments result in greater between-population variation? This might be expected because different populations may by chance adapt to different niches, or because greater within-population diversity (resulting from selection in the complex environment) increases the probability of significant epistatic effects. The answer appears to be yes. The study of *A. eutrophus* revealed greater differences in fitness between populations evolved in spatially heterogeneous than spatially homogeneous environments.⁽¹⁷⁾ Similarly, greater between-population morphological diversity was observed when *P. fluorescens* evolved in spatially heterogeneous compared with spatially homogeneous environments, and when the environment is made more complex by the presence, compared with absence, of parasitic bacteriophage.⁽³⁹⁾

Thus there is some strong empirical support that environmental complexity plays an important role in moulding adaptive landscapes. However, we must regard these conclusions with care since there is a danger of circularity. If we define complex landscapes as those that contain multiple niches, but only recognise multiple niches when there are alternative adaptive peaks, then we are in danger of defining complex environments based on their adaptive landscapes.

Conclusions

Microbial studies are beginning to provide insights into the nature of adaptive landscapes and the way in which such landscapes can affect the path of adaptation. So far the results suggest that adaptive landscapes are far from smooth and that, even within a simple laboratory environment, the potential genetic solutions to the same evolutionary problem may be many. That this seems to be the case, even in genetically simple organisms (the virus phi6 genome only encodes 13 proteins) suggests that rugged landscapes will be the rule rather than the exception. In practical terms, rugged landscapes increase the unpredictability of the evolutionary process. In times of unprecedented anthropogenic changes to the environment, this will make prediction of the long-term consequences of our actions difficult at best. The lessons of antibiotic resistance are clear.

Will these findings apply more generally to organisms other than microbes? In particular, these experiments were generally done with large asexual populations. There are reasons to believe that both of these factors may make adaptive landscapes smoother. Large populations will have large numbers of mutations arising in every generation. This essentially means that they are constantly sampling large areas of the surrounding fitness landscape, and even if multiple peaks

of different height exist, they are likely to “find” the highest peak, before the whole population becomes fixed on a suboptimal peak.

Asexuality may further affect this process. For an asexual population ascending a suboptimal adaptive peak to move to the highest adaptive peak, all that is required in principle is a single mutant to arise within the attractor of the higher peak. In a sexual population, even if such a genotype is produced, it may be rapidly destroyed again by recombination. On the other hand, a sexual population can also produce such a genotype by recombination. Thus whether a sexual or an asexual population is more likely to be constrained depends on the details of the landscape, in particular the relative heights of the different peaks, and the depths of the valleys.⁽⁴¹⁾ Experimental evolution studies that directly compare asexual and sexual microbes, for example using facultatively sexual organisms such as *Chlamydomonas* spp. and *Saccharomyces* spp., would provide key insights into this issue.^(42,43)

None of these studies have included the kinds of temporal environmental change that is typical of other systems, and may cause the topology of landscapes to change dramatically and unpredictably thorough time. If real adaptive landscapes are typically more like turbulent oceans than fixed alpine ranges they may offer little constraint to the adaptive process,⁽⁴⁴⁾ even though experiments carried out in constant environments suggest otherwise. Microbial experiments carried out in changing environments would provide important insight into this issue.

Nevertheless, the fact remains that evidence is found for rugged adaptive landscapes, even in these very simple systems. When combined with evidence from other sources,^(4–6) this implies that such structure may be ubiquitous and play an important role in the evolutionary process of all species.

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