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Review Article

Dynamics of bacterial adaptation

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Determining pattern in the dynamics of population evolution is a long-standing focus of evolutionary biology. Complementing the study of natural populations, microbial laboratory evolution experiments have become an important tool for addressing these dynamics because they allow detailed and replicated analysis of evolution in response to controlled environmental and genetic conditions. Key findings include a tendency for smoothly declining rates of adaptation during selection in constant environments, at least in part a reflection of antagonism between accumulating beneficial mutations, and a large number of beneficial mutations available to replicate populations leading to significant, but relatively low genetic parallelism, even as phenotypic characteristics show high similarity. Together, there is a picture of adaptation as a process with a varied and largely unpredictable genetic basis leading to much more similar phenotypic outcomes. Increasing sophistication of sequencing and genetic tools will allow insight into mechanisms behind these and other patterns.

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In 1944 Simpson published *Tempo and Mode in Evolution* [1]. This book was an attempt to understand the dynamics of adaptation — how biological populations evolve as they are selected in a given environment to increase in fitness. Which traits change and how quickly? Do changes occur at a constant rate, follow some other predictable pattern, or are they variable? How rapid is the divergence between initially identical populations selected subject to some barrier that causes their evolution to be independent? In short, what aspects of evolution can, at some level, be understood and even predicted?

We present an overview of the dynamics of adaptation as they impact repeatability and predictability. We focus on studies that use simple bacterial systems without the added complication of horizontal gene transfer. The degree of control possible in these systems, especially the ability to begin experiments with initially identical replicate populations, is crucial for separating the influence of chance and deterministic factors in evolutionary outcomes. Throughout, we define adaptation as the evolutionary process through which organisms become better fitted to their environment. Adaptive dynamics, therefore, describes the pace and form of the process of adaptation.

Dynamics of adaptation depend on the underlying fitness landscape

Fitness landscapes were introduced by Wright nearly 90 years ago to illustrate the dynamic process of adaptation [2] (Figure 1). The metaphor imagines a two-dimensional surface on which similar genotypes are close to one another and can be transitioned between by mutational changes. Onto this landscape a fitness surface is projected, where peaks indicate genotypes of high fitness and valleys genotypes of low fitness. Whereas mutation moves populations randomly on the two-dimensional genotype surface, natural selection biases movement toward genotypes of higher fitness because these genotypes, by definition, tend to leave more offspring. The dynamics of evolution therefore depends on the form of the landscape. For example, the number of peaks available through uphill paths from a starting position will affect the predictability of evolution and the steepness of the slopes leading to them influences the rate of adaptation.

The fitness landscape is limited in that it serves as a simplifying metaphor of a much more complicated process. Most obviously, relationships between genotypes are of very high dimension, whereby any given genotype can be one mutational change away from many others with each of these being a

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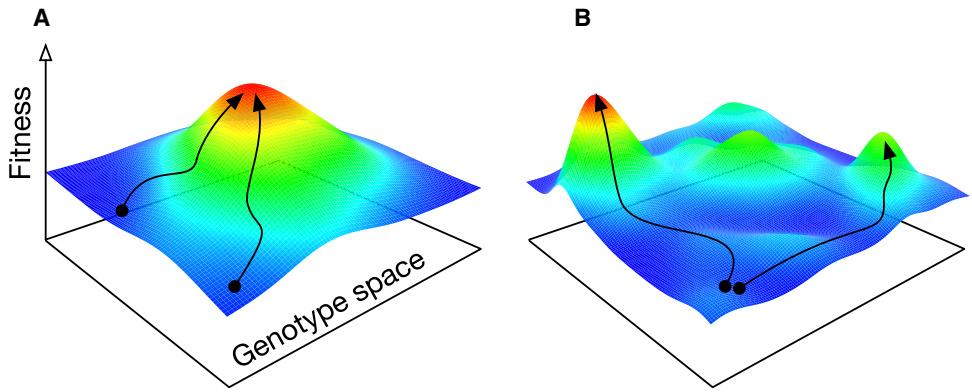

Figure 1. Fitness landscapes represent evolutionary possibilities available to evolving populations.
Landscapes define the relationship between genotype and fitness, revealing the availability of 'uphill' fitness paths available to be followed by populations through the action of natural selection. (**A**) A smooth fitness landscape has many available paths leading to a global fitness peak. Independently evolving populations will tend to converge to the same genotype and fitness, even if started from different initial positions (genotypes) on the landscape (arrows). (**B**) A rough fitness landscape can have multiple fitness peaks so that populations will tend to diverge to different peaks dependent on their starting position. Even populations starting from the same position might diverge as chance differences are built on (arrows). We emphasize that the landscape is a metaphor for the much more highly dimensional interactions available to real organisms.

stepping-stone to many more possibilities. Nevertheless, the fitness landscape provides a valuable way to intuitively describe and visualize adaptation. Throughout this review, we use the landscape as an organizing principle, referring to it to help illustrate processes underlying particular evolutionary patterns.

Evolutionary randomness and pattern are not exclusive

Understanding the tempo of evolutionary change has long been a focus of evolutionary biology. For features that fossilize, it is possible to track changes over time. That work, while being enormously valuable, is limited in being retrospective, reflecting what has happened over long timescales in natural environments. As such, it is difficult to account for the influence on the evolution of complicating factors such as environmental change and migration between populations. A big advantage of studying laboratory evolved populations is the ability to control such factors, offering the opportunity to precisely track dynamics in known conditions. As well, of course, it is possible to track the dynamics of phenotypes, like fitness, that do not fossilize.

Laboratory evolution also offers another less obvious advantage. The ability to observe the evolution of replicated populations means that adaptative outcomes can be described statistically, including some measure of the range of different outcomes that can occur. This is important because adaptation is influenced by chance, depending on the inherently stochastic production and selection of genetic variation [3,4]. For example, on 'rugged' fitness landscapes that have multiple peaks, populations starting at the same place can diverge to different fitness peaks if different sequences of mutations arise (Figure 1B). Notwithstanding the effect of this stochasticity, it is nevertheless possible that the impact of the mutations that occur and are selected will lead to some repeatable pattern in the evolution of important traits, such as fitness. Indeed, many mutations are likely to affect fitness, so a population's rate of fitness change might depend more on how many relevant mutations are substituted, less on their exact identity. In short, analysis of replicated evolving populations allows for changes to be described as a pattern, rather than idiosyncrasy.

Patterns of fitness change in simple environments

An experiment (the Long-Term Evolution Experiment (LTEE)) allowing examination of the dynamics of adaptation to a simple constant environment was begun by Richard Lenski in 1988 [5]. He founded 12 replicate populations with a single strain of the *Escherichia coli* bacterium and selected each in a common defined environment. One of the initial motivations for the experiment was to describe the pattern of adaptation. To do this, Lenski and colleagues periodically estimated the fitness of evolved populations relative to the ancestor and analyzed the trajectories of its change [5]. These trajectories represent the rate of change along the vertical

fitness axis as populations traverse the fitness landscape and can reveal both the fact of differences in the underlying genetic changes and some aspects of the nature of those changes. For example, whether accumulated mutations tend to interact to cause fitness increases to be less than expected from individual effects.

At the start of the experiment, several possibilities for patterns of fitness change were imagined. It could be saltational, proceeding in 'steps' as rare beneficial mutations arose and increased in frequency within each population, or gradual, depending on the action of many contributing mutations combining to smooth out their individual effects. In either case, fitness could approach an optimum at a constant rate or one that slowed over time [5].

Analysis of fitness trajectories in the LTEE showed clear support for a smooth increase in fitness occurring at a declining rate [6]. Both observations reflect and help illuminate complicated underlying processes. The smooth change in fitness is expected if beneficial mutations are relatively common so that they are constantly arising in adapting populations [7]. Because the bacteria in the evolving populations had no means to recombine mutations occurring in separate lineages, they must compete with one another. Before that happens, additional mutations can occur, leading to a complex nested set of competing genotypes and an overall smooth increase in fitness as individual mutation effects are diluted by the effect of competition between mutations. Subsequent genetic analysis has confirmed the outline of this process [8].

Perhaps more surprisingly, the rate of fitness change declined quickly over time [9]. Several non-exclusive explanations have been considered to explain this result [9–11]. It could be that populations initially had access to a finite pool of mutations of fixed effects, i.e. effects that did not depend on the order in which the mutation occurred. In that case, populations would be expected to adapt most quickly during the early part of their evolution as more of the larger benefit mutations remained available to be substituted, leading to both a higher beneficial mutation rate and a higher mean effect of substituted mutations. A second kind of explanation involves the action of 'contingency' a phenomenon whereby mutation effects change through the course of the adaptation of a population. This change arises when the effect of a mutation depends on the particular genetic background in which it occurs. Specifically, if the fitness benefit conferred by a mutation declined in proportion to the fitness of a population, a declining rate of adaptation would be expected. This kind of pattern has been seen, for example, in mutations that compensate for the costs of antibiotic resistance [12–15].

To distinguish between these possibilities, Wiser et al. (2013) analyzed the pattern of fitness increase over the first 20 000 generations of the LTEE. They considered a number of models to do this, including a power law model that predicts future fitness increase as a function of current fitness. (This kind of relationship is a special example of contingent mutation effects called diminishing-returns epistasis that predicts that new beneficial mutations confer a benefit that decreases proportional to the fitness of the strain they occur in [16]). This power law model was found to fit the data well and, crucially, to also be able to predict fitness change over the *next 30 000* generations, despite that data not having been considered in developing the model. A subsequent experimental test that directly determined the effect of newly arising mutations measured in the (high-fitness) strain they occurred in and in the (lower fitness) ancestor was consistent the power-law model. Analysis of fitness evolution and mutation effects in other model systems have been largely consistent with this pattern indicating that it is likely reflects some general mechanistic process of adaptation to constant environments [17–19].

Repeatability of replicate evolutionary trajectories

An important motivating question in microbial evolution studies has been the extent to which adaptation is repeatable. That is, if evolution could be repeated from the same genetic starting point and subject to the same selection, would outcomes be similar? This idea was famously captured by Stephen Jay Gould's thought experiment imagining a replay of the tape of life. In Gould's words: 'I call this experiment "replaying life's tape." You press the rewind button and, making sure you thoroughly erase everything that actually happened, go back to any time and place in the past… Then let the tape run again and see if the repetition looks at all like the original [20]' While noting that the experiment, as envisioned, was impossible, Gould was nevertheless of the view that evolutionary outcomes are unlikely to be repeatable.

Gould's reason for this conclusion was that mutations interact with one another so that the effect of a given mutation is contingent on which other mutations have already occurred in a particular evolving population. The diminishing-returns epistasis described above is one example of a contingent mutation interaction, but other more dramatic examples are well known. For example, some mutations can compensate for previously occurring deleterious mutations, restoring original function, but have no effect if considered on their own [21]. Because mutation occurrence is subject to stochasticity, so must be the outcome of evolutionary processes.

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With reference to the fitness landscape, contingency causes initial differences in population movement on the fitness landscape, representing different genotypes, to alter the effect of possible new genetic mutations. In practice this makes accessible new regions of the landscape, increasing the likelihood of populations following divergent trajectories to distinct fitness peaks.

Microbial laboratory evolution experiments allow Gould's idea to be tested. When replicated populations evolved in simple environments are compared, repeatability of fitness change can be high [9,22]. With clever experimental designs, it is even possible to determine the influence of different kinds of factors on the divergence of population fitness. In one classic study, replicate populations were first evolved in one environment, then transferred to a second and evolved further with additional replication [23]. This design allowed the basis of fitness divergence after the second period of evolution to be partitioned between dependence on differences that occurred after the first period of evolution (i.e. 'history') and stochastic mutation occurrence and selection between replicate populations (i.e. 'chance'). History and chance effects had almost no influence, consistent with a strong tendency for convergence in fitness. This convergence is consistent with a landscape on which the populations started with and retained access to a single fitness peak so that chance differences in mutation occurrence do not become influential. Nevertheless, it is important to emphasize that differences between replicate populations can arise. The best studied is probably the origin of the ability to grow on citrate in one of the LTEE populations, which has been found to depend on an interacting set of mutations [24,25].

Many studies have focussed on examining adaptive dynamics at the level of fitness because, in laboratory-evolved populations, it can be easily measured and is fundamental to determining evolutionary outcomes. However, changes in fitness depend on a vast number of lower-order cell processes and it may be that different underlying genetic, and perhaps molecular-level phenotypes (e.g. gene transcription or translation levels), might lead to similar fitness effects. If so, parallel evolutionary dynamics at the level of fitness might obscure divergent changes at other levels. One comprehensive test of this possibility comes from the LTEE. Sequencing of evolved populations found a large number of mutational changes [8,26]. Mutations were distributed approximately evenly between 57 genes that were mutated in more than two of the 12 populations and other genes mutated in a single population. This, significant, but far from complete, parallelism is consistent with there being many possible genetic solutions to increase fitness in the selective environment. Detailed transcriptional and translational analysis of the consequences of the mutations, however, indicates extremely high parallelism of these phenotypes, consistent with a pattern of different genetic routes to similar molecular-level phenotypic changes causing similar fitness changes [27].

A diversity of genetic routes to a similar higher-order phenotype has also been seen an experiment examining the pathways through which the bacterium *Pseudomonas fluorescens* can evolve the ability to switch colony morphologies or to evolve the ability to colonize a new ecological niche [28–30]. The genetic pathways underlying niche colonization were analyzed in a detailed mechanistic model that aimed to predict the likelihood that each would be followed [31]. This model was broadly successful, but also revealed the influence of unanticipated mutational hotspots. In effect, these hotspots biased movement on the fitness landscape so that mutation success, that is, movement on the landscape, couldn't be predicted only from their fitness effects.

An effect of plasmids on bacterial adaptive dynamics

The work described above focusses on dynamics of adaptation of asexual bacterial populations evolving in the absence of any additional genetic elements. This represents a simplest case where underlying evolutionary possibilities are influenced by the fitness landscapes determined by the effects of genetic interactions occurring within single chromosomes. We finish by considering one additional layer of complexity: the influence of bacterial plasmids on adaptive dynamics. Plasmids can affect the fitness landscape by altering the relationship between bacterial genotypes and their fitness in a way that makes plasmids more likely to persist.

Bacterial plasmids are accessory genetic elements that are not usually necessary for the growth of their bacterial host in at least one environment [32]. Nevertheless, plasmids are common in natural populations and many plasmids encode conditionally beneficial genes that are selected in some environments — for example antibiotic resistance genes that provide an advantage in environments containing the cognate antibiotic — so their effect on evolutionary dynamics can be significant and are important to consider [33–35].

A key consideration in predicting the influence of plasmids on adaptive dynamics of bacterial populations is the cost that they impose on host cells in the absence of direct positive selection for the genes they carry [36]. This cost helps determine the persistence of plasmids in environments in which they do not provide a direct advantage, and thus the opportunity for the genes they carry to contribute to bacterial fitness when the

environment changes [32,37]. Importantly, this cost is not fixed, it can be reduced by 'compensatory' mutations that help maintain plasmids by decreasing their cost relative to plasmid-free cells [38,39]. Positive selection for plasmids can be rare in natural environments, yet plasmids are nevertheless common in bacterial populations [34]. Apart from the transmissive ability of conjugative plasmids, one explanation for this apparent discrepancy is the action of compensatory mutations. Indeed, studies have begun to show the importance of the interplay between selection and compensatory mutations on the adaptation of plasmids and their hosts.

An example of the interplay of positive selection and compensatory mutations on adaptation comes from an experiment conducted by San Millan et al. [30] (Figure 2). Using a non-conjugative plasmid — thus removing the complication of plasmid transfer between cells — they demonstrated that rare positive selection for plasmids can enhance their persistence via an interaction between specific mutations that compensate for plasmid costs and mutations that contribute generally to adaptation. When a plasmid-carrying population evolves in the absence of selection for the plasmid, plasmids are expected to be gradually lost from the population as a result of occasional plasmid missegregation during cell replication combined with the cost imposed by plasmids on host cells. San Millan et al. (2014) found that a brief pulse of positive selection for a plasmid increases its long-term persistence. Counterintuitively, the longer a population goes without experiencing this pulse, the more likely the plasmid will persist. This effect was explained by the action of compensatory mutations arising in plasmid-carrying subpopulations that increase to a high frequency after the pulse of plasmid selection. Furthermore, the selection sweep resets the frequency of the plasmid-carrying subpopulation to 1, increasing the chance that generally adaptive mutations occur and support plasmid persistence. In summary, rare positive selection events together with the availability of compensatory mutations provide pathways in the fitness landscape to allow selection of reduced plasmid costs and may help explain the ubiquity of plasmids in bacterial populations.

Though studies have begun to reveal the roles of selection and compensatory evolution on the evolution of plasmids, there remain many unanswered questions. For example, the location of compensatory mutations on plasmids or chromosomes can theoretically lead to either a plasmid able to persist in different hosts or a host able to generally tolerate a range of plasmids [38,39]. In the study of San Millan et al. 2014, the compensation

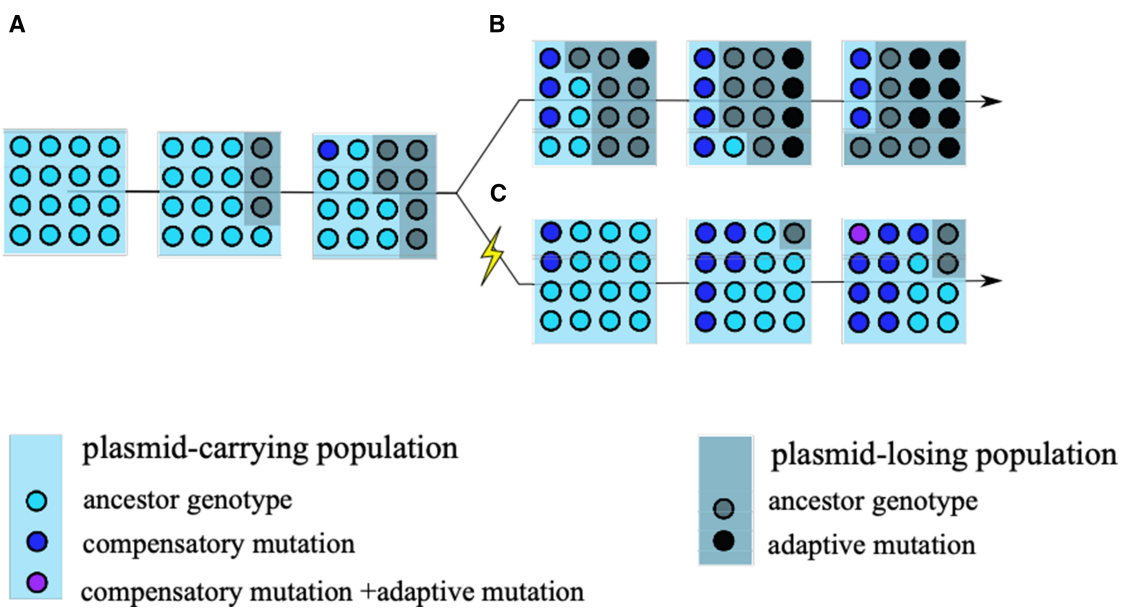

Figure 2. Interaction between positive selection and compensatory mutations can prolong plasmid stability.
(**A**) Without positive selection plasmids are gradually lost from a population (squares containing individual cells (circles)) due to missegregation and slower growth of plasmid-carrying cells. Compensatory mutations reduce the cost of plasmid carriage, increasing its persistence, but missegregation will lead to eventual extinction in the absence of plasmid transmission. (**B**) This extinction will be hastened if adaptive mutations that increase cell fitness occur in the plasmid-free subpopulation, thereby increasing the relative fitness of plasmid-free to plasmid-carrying cells. (**C**) A pulse of positive selection for the plasmid resets its frequency in the population to one, increasing the chance that any new adaptive mutations occur in the plasmid-carrying subpopulation.

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can come from mutations in a host DNA helicase. Compensation via host DNA helicases has been observed in different plasmids [39], suggesting that compensatory mutations to one plasmid may provide compensation to another. Moreover, the prevalence and effect of compensatory mutations in natural populations is not well understood. Such knowledge would provide a deeper understanding of, for example, how antibiotic-resistant plasmids circulate in nature where positive selection may be weak and rare.

Perspectives

- Microbial experimental evolution studies have allowed detailed examination of both the pattern of evolutionary dynamics and of the basis of those patterns.
- Several general patterns of adaptive dynamics are consistent across different experimental systems. In contrast, the genetic basis of adaptation can be variable, even across replicate populations evolved in the same experiment.
- There has been a focus on examining adaptive dynamics in 'simple' conditions, often starting experiments with clonal populations selected in constant environments. Ongoing challenges include building on studies considering the effect of sources of genetic and environmental complexity on adaptive dynamics and determining the physiological and genetic basis of evolutionary patterns—for example, the basis of the pattern of diminishing-returns epistasis.

Competing Interests
The authors declare that there are no competing interests associated with the manuscript.

Author Contributions
The authors contributed equally to all aspects of the preparation of this manuscript.

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Abbreviations
LTEE, long-term evolution experiment; DNA, deoxyribonucleic acid.

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