

Genomic investigations of evolutionary dynamics and epistasis in microbial evolution experiments

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Microbial evolution experiments enable us to watch adaptation in real time, and to quantify the repeatability and predictability of evolution by comparing identical replicate populations. Further, we can resurrect ancestral types to examine changes over evolutionary time. Until recently, experimental evolution has been limited to measuring phenotypic changes, or to tracking a few genetic markers over time. However, recent advances in sequencing technology now make it possible to extensively sequence clones or whole-population samples from microbial evolution experiments. Here, we review recent work exploiting these techniques to understand the genomic basis of evolutionary change in experimental systems. We first focus on studies that analyze the dynamics of genome evolution in microbial systems. We then survey work that uses observations of sequence evolution to infer aspects of the underlying fitness landscape, concentrating on the epistatic interactions between mutations and the constraints these interactions impose on adaptation.

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Introduction

Laboratory evolution experiments complement studies of natural populations, by making it possible to more directly investigate the underlying factors that shape observed patterns of genetic diversity. While these experiments neglect many important complexities of the natural world, they offer a number of advantages. In experimental systems, we can observe evolutionary dynamics in real time, and exploit a ‘frozen fossil record’ to resurrect ancestral types and directly compare them to their descendants. We

can also maintain many populations in parallel, and replay the tape of life thousands of times in identical (or different) conditions. Finally, we can tune key evolutionary parameters such as population sizes and mutation rates, and assess their importance with other parameters held constant.

A large body of work has exploited these advantages to investigate how evolutionary history, chance, and natural selection influence evolutionary outcomes [1,2]. For example, recent work has examined how the distribution of fitness effects of available mutations determines the power of natural selection [3,4]. Additionally, multiple studies have analyzed the role of epistasis in creating ‘historical contingency,’ where an initial mutation constrains or potentiates future evolution [5^{*},6,7]. Finally, laboratory evolution has been used to investigate how parameters such as population size affect evolutionary dynamics [8,9]. All of these studies aim to describe how features of the evolutionary landscape combine to determine evolutionary outcomes — a general process that is not confined to laboratory systems. When used for this purpose, laboratory microbial evolution experiments have the potential to be a model system for understanding the structure and diversity of genomes.

Until recently, experimental evolution has been limited primarily to phenotypic measurements. Numerous studies have examined how fitness changes over time, and how the rate of adaptation depends on factors such as the population size, initial genotype, population structure, or environmental conditions. Many studies have also tracked the frequencies of observable markers (e.g. drug resistance or fluorescent reporters) through time [3,4,10,11] to draw inferences [3,12,13] about the evolutionary process. In recent years, however, advances in sequencing technology have made it possible to sequence clones or whole-population samples from hundreds of parallel experimental lines [14–16]. In this review, we summarize recent work that has begun to apply these technical advances to long-term laboratory evolution experiments, to directly observe how microbial genomes evolve in the laboratory. We focus particularly on the influence of epistasis on genome evolution (for a more general and comprehensive review, see [17]).

The dynamics of adaptation

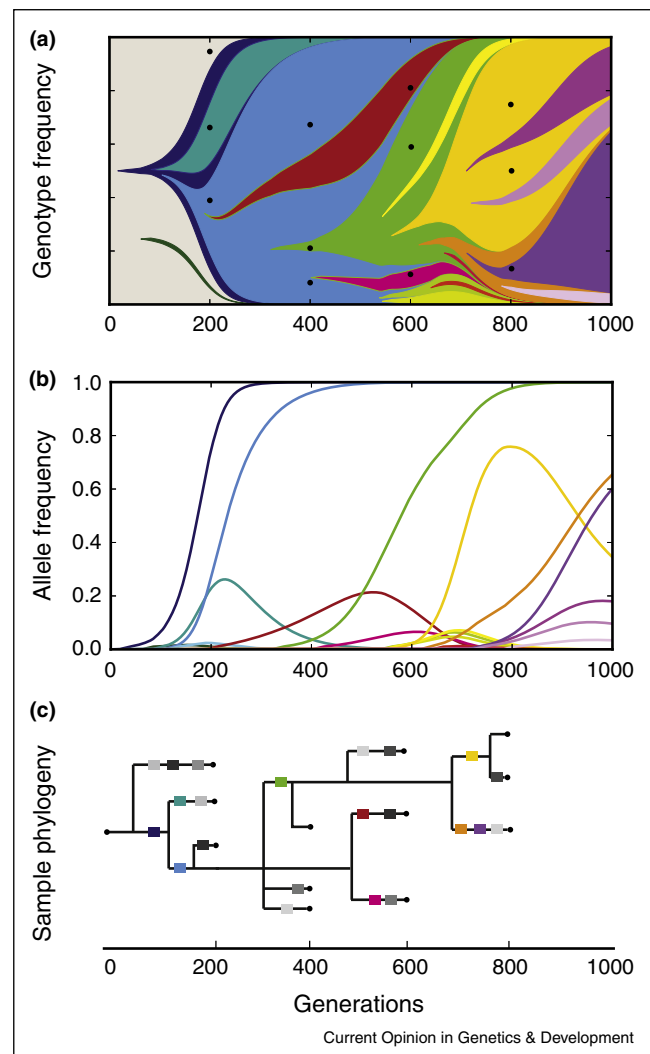
The population genetic forces of mutation, selection, and drift govern the dynamics of genome evolution, determining which mutations will survive competition to fix in

the population, and the signatures this process will leave on patterns of genetic diversity. An extensive population genetics literature has explored these connections theoretically. However, until recently it has been impossible to directly observe these dynamics in laboratory populations. Empirical studies of this type are necessary to determine which theoretically proposed regimes evolving populations actually experience. For example, is adaptation typically mutation limited, and hence in a regime where beneficial mutations fix independently, on their own merits? Or do beneficial mutations typically arise in multiple different lineages, resulting in a 'clonal interference' regime where many mutations compete for dominance? Studies that directly observe genome evolution in real time can distinguish between these and other possibilities. They can also point the way to complications that theory has overlooked, but that may be essential to the evolutionary process.

The first experiments to directly observe genomic evolution were in bacteriophage, where several studies Sanger-sequenced individual phage clones at multiple timepoints in several replicate populations to describe patterns of parallel and convergent evolution [18–23]. Next-generation sequencing now makes similar studies possible in microbial populations. Recent studies have exploited both whole-population ('metagenomic') sequencing of samples isolated at multiple timepoints from one or more evolving lines, and also sequencing of clones, to understand genomic evolution in these populations. These approaches offer somewhat different perspectives on the dynamics of molecular evolution (Figure 1).

The first major study to examine the dynamics of genome evolution in microbial laboratory populations focused on a single line of a long-term evolution experiment in *Escherichia coli*. By analyzing a clone sampled from each of five timepoints during 20,000 generations of adaptation, Barrick *et al.* [24**] showed that mutations continue to accumulate steadily through time despite a dramatic slowdown in the rate at which fitness increases. This divergence between phenotypic and sequence-level evolution points to the potential importance of epistasis in shaping adaptation [25–27]. More recent studies have sequenced clones isolated from multiple timepoints in several replicate populations [28,29]. This work demonstrates that adaptation is typically not mutation-limited: instead, there is clonal interference between competing beneficial mutations. Clonal interference affects which mutations fix in the population, and hence influences both molecular diversity and the dynamics of adaptation. In particular, the competition among beneficial mutations ensures that many are wasted, reducing the efficiency of selection and the predictability of molecular evolution. Clonal interference also affects the efficacy of other evolutionary processes, such as indirect selection on mutation rates [30].

Figure 1



Schematic of the evolutionary dynamics in a microbial evolution experiment. **(a)** Muller diagram depicting the frequencies of each genotype in the population over time. Only lineages that reach substantial frequency are shown (many lower-frequency lineages will typically also exist). **(b)** Allele frequencies in the population from (a), as they would be measured using whole-population metagenomic sequencing. This strategy reveals the dynamics of major alleles, but low-frequency mutations are undetectable. This metagenomic data also yields incomplete haplotype information: it is not always clear which mutations arise on which genetic backgrounds. **(c)** A phylogenetic tree built from clones that could be sampled from the population in (a; black dots). Colored boxes show the major mutations pictured in (a) and (b); grey boxes show 'private' mutations shared only by this clone and close relatives. This clone sequencing approach can be used to measure mutation rates and genetic diversity statistics such as heterozygosity, but provides limited information about allele frequencies over time.

Sequencing whole-population samples through time in multiple replicate populations offers an alternative view of the dynamics of adaptation [31**,32,33]. For example, one large-scale study in budding yeast highlighted the

importance of pervasive clonal interference and hitchhiking in determining the outcomes of adaptation and in limiting the degree of parallelism in the genome evolution of replicate lines [31^{••}]. Other studies have shown that frequency-dependent interactions can evolve spontaneously and play an important role in both lineage dynamics and the maintenance of genomic diversity [34,35[•]]. Finally, while most laboratory evolution experiments start from clonal populations, and hence focus entirely on evolution from new mutations, another body of work has used whole-population timecourse sequencing to analyze the dynamics of adaptation from standing genetic variation. Much of this work is carried out in *Drosophila* [36–38], but recent work has addressed similar questions using extensive sequencing of yeast populations founded from outcrossed lines [39[•]].

Epistasis, historical contingency, and the structure of the landscape

Genome evolution is shaped both by the dynamics of the evolutionary process and by the spectrum of available mutations and their fitness effects (i.e. the ‘fitness landscape’). Thus we can use observations of genome evolution as a tool to infer relevant aspects of the landscape. Here we focus on recent progress in characterizing patterns of epistasis (i.e. how the fitness effects of mutations depend on the genetic background), and in understanding how epistasis constrains evolution. In principle, acquiring one mutation can change the entire distribution of fitness effects of future mutations. This ‘macroscopic epistasis’ summarizes many underlying details of individual genetic interactions [26], and can lead to historical contingency where an initial mutation constrains the future course of adaptation. Even without extensive macroscopic epistasis, interactions among specific sets of mutations (‘microscopic epistasis’) can affect the mutational trajectories open to an evolving population, and hence lead to historical contingency in patterns of molecular evolution. Microbial experimental evolution is particularly well-suited to probing patterns of both macroscopic and microscopic epistasis, because replicate populations will probabilistically explore available areas of the local landscape, and mutations that arise during evolution can be reconstructed in arbitrary combinations. Extensive replication also makes it possible to conduct large-scale studies of the mutations that occur during evolution and their statistical associations with one another, providing another type of evidence for epistasis [40].

Recent work has explored whether patterns of epistasis reflect the modularity of cellular organization. Within the smallest modular unit, a single gene, studies of the antibiotic resistance enzyme beta lactamase [41^{••}] and comprehensive reconstructions of mutations within a nine amino acid region in yeast Hsp90 [42,43] have revealed widespread epistasis. This includes sign epistasis, in which a mutation shifts from beneficial to

deleterious (or vice versa) depending on the genetic background it occurs in. Epistatic interactions may also occur at the protein complex or pathway level. In a landmark paper, Tenaillon *et al.* [44^{••}] demonstrated such an effect in *E. coli* adapting to heat stress. By evolving 114 replicate populations and sequencing their genomes after evolution, this study uncovered two mutually exclusive routes to heat adaptation: populations acquired mutations either in the RNA polymerase pathway or in the termination factor rho, but not both [44^{••},45] (see also [28] for a related effect). Further, in a single line there were often multiple mutations in one or the other pathway. To explore epistasis at the pathway level more broadly, two recent studies in *E. coli* and yeast evolved replicate populations with particular gene deletions, and found an enrichment in compensatory mutations targeting complexes associated with the deleted gene [46,47]. Together these studies indicate that a mutation in a particular pathway, either beneficial or deleterious, may potentiate beneficial mutations in this pathway. By contrast, within a single gene, one mutation is more likely to exclude further changes.

Several recent studies have found indications of a different, broader form of epistasis. In viruses, bacteria, and yeast, fitter genotypes reliably adapt more slowly than less-fit genotypes [5[•],27,48–51]. This slowdown indicates that the distribution of fitness effects of available beneficial mutations depends on genotype, either because there are fewer beneficial mutations available to fitter genotypes, or because the beneficial mutations have weaker effects (‘diminishing returns epistasis’). Studies in *E. coli* and methylobacterium reconstructed all combinations of four or five mutations from an adaptive walk, finding a general pattern of diminishing returns [52,53]. More recently, Kryazhimskiy *et al.* [5[•]] used 640 replicate yeast populations to show that the rate of adaptation declines systematically with the fitness of the initial genotype — not because more-fit populations run out of beneficial mutations, but rather because the effects of beneficial mutations become weaker in fitter backgrounds. This effect is largely independent of the specific mutations involved, indicating a potentially general negative coupling among beneficial mutations.

Occasionally more dramatic effects of epistasis arise, where a mutation potentiates a major shift in the DFE, sometimes including changes to the population structure. The most extensively studied example of such an effect is from Richard Lenski’s long-term evolution experiment in *E. coli*. In one of 12 replicate lines, after about 30,000 generations one lineage evolved the ability to utilize citrate under aerobic conditions [6]. This lineage stably coexisted for thousands of generations with the wild-type Cit[−] lineage, indicating the presence of frequency-dependent selection. Recent work has described the genetic basis of this transition, including multiple potentiating

mutations [54^{••},55]. While this example is so far unique, the effects of epistasis in potentiating the evolution of coexisting lineages have also been observed in another replicate population from the same experiment [56]. In both of these cases, historical contingency was essential: multiple interacting mutations are required to create the new phenotype. Related examples where sign epistasis limits potential evolutionary trajectories, leading to important effects of historical contingency, have been described in other systems [57,58].

Larger-scale genomic changes, including shifts in ploidy, amplifications, deletions, inversions, copy number variation, and transposon-mediated insertions, can also represent an important aspect of the fitness landscape. Because of the limitations of short-read sequencing technology, these events have been comparatively overlooked in many laboratory evolution experiments [59]. However, they are a potential avenue for rapid innovation [59–62], and can clearly have dramatic effects on the future course of adaptation (e.g. they can lead to substantial macroscopic epistasis). The rates at which these events occur and the selective forces that act on them remain incompletely understood. For example, ploidy is clearly tuned rapidly by natural selection [63]. However, while recent studies find that experimental budding yeast populations quickly converge towards diploidy, these changes do not always have clear selective advantages [64–68]. In addition to changes in ploidy, amplifications or deletions of individual chromosomes (or parts of chromosomes) can also be rapidly selected, particularly in stressful conditions [69[•],70,71,72[•],73,74] or when starting from novel genotypes [75,76]. These amplifications or deletions can change the local fitness landscape, opening new avenues for adaptation. For example, a study of yeast adapting to high temperature [69[•]] demonstrated that the initial phases of adaptation often involve a duplication of chromosome III. This duplication eventually reverts, and is replaced by a more ‘refined’ solution that upregulates only specific genes on this chromosome. Copy number variation can also provide a rapid avenue to dosage adjustment. For example, studies of yeast in a variety of nutrient-limited conditions have found adaptive amplifications of transporters of the limiting nutrient [32,73,74].

Conclusions and future directions

Experimental evolution makes it possible to observe genome evolution in real time in a controlled, replicated laboratory setting. It also allows us to directly measure (and in some cases control) the underlying evolutionary parameters that influence which mutations fix and hence how genomes evolve. With the advent of inexpensive high-throughput sequencing, it has become possible to examine the dynamics of new mutations within adapting populations in unprecedented detail. Although experimental systems cannot reproduce the complexities of natural populations, the same rules of population genetics

govern evolutionary dynamics in both systems, and so the dynamics observed in laboratory populations may be common to more complex natural cases. For example, recent studies have demonstrated the prevalence of clonal interference in microbial laboratory populations; this regime may be common in natural systems, such as *Pseudomonas* infections in the lungs of cystic fibrosis patients [77]. Recently, studies have also begun moving beyond the typical isolated population of laboratory evolution, to examine the emergence and maintenance of population structure [34,35[•],78–85]. In these studies, frequency-dependent selection gives rise to stable ecotypes readily even in very simple laboratory environments. Understanding the prevalence, dynamics, and long-term fate of these structured populations will help bridge the gap between the rich ecology of the natural world and the simplicity of laboratory evolution.

Recent progress has also been made towards understanding the structure of the fitness landscape, which plays a key role in determining evolutionary dynamics and patterns of genomic diversity. In particular, recent work has shown that diminishing-returns epistasis (where beneficial mutations generally damp each others’ effects) may be a general feature of adaptation. Studies have also begun to examine how cellular organization influences the mutations that fix during evolution. Future work will probably bring increasing power to understand the fitness landscape and the cell via statistical associations between mutations that fix during evolution. New approaches, such as massive barcoding systems [86^{••}], also promise to provide a yet more detailed view of evolutionary dynamics, and also to significantly increase power to understand epistasis. These systems offer the prospect of measuring the dependence of the distribution of fitness effects on both genotype and environment, as well as the interactions between individual mutations.

To date, most microbial laboratory experiments have used model organisms, notably *E. coli* and *Saccharomyces cerevisiae*. While the tractable genetics in these systems provides a strong advantage, in the future it will also be important to investigate the evolutionary process in a wider variety of organisms. This breadth will be necessary to help us distinguish which features of the evolutionary process are general, rather than specific to common laboratory models. Early progress has been made in describing mutational processes [87–89] and evolutionary outcomes [35[•],76,90] across a broader variety of microbes. Further studies along these lines will provide a more comprehensive view, and help connect with clinical and industrial applications where specific non-model microbes may be of particular interest.

Finally, the last few years has seen an explosion of data on microbial communities in nature and their roles in animal and plant health. However, relatively little is known

about evolution in these complex environments. Studying evolution in these scenarios, and the interplay between the host and the community, is an exciting avenue of future research (e.g. see [29] for a pioneering study).

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