

Experimental evolution in an era of molecular manipulation

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Abstract

Laboratory evolution experiments in microbial and viral populations have provided great insight into the dynamics and predictability of evolution. The rise of high-throughput sequencing technologies over the past two decades has driven a massive expansion in the scale and power of these experiments. However, until recently our abilities to connect genetic with phenotypic changes and analyse the molecular basis of adaptation have remained limited. Rapid technical advances to measure and manipulate both genotypes and phenotypes are now providing opportunities to investigate the genetic basis of phenotypic evolution and the forces that drive evolutionary dynamics. Here we review how these methodological advances are being used to predict and manipulate the course of laboratory evolution, analyse eco-evolutionary interactions, and how they are beginning to bridge the gap between laboratory and natural evolution.

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Introduction

All the diversity of the biological world – the disparate forms and functions we find across organisms – has been generated by evolution. As evolutionary processes typically occur over long timescales, evolution has historically been studied almost exclusively retrospectively, inferring past evolutionary dynamics through palaeontology or population genetics. These approaches have provided fundamental insights into many aspects of evolution, including the importance of stochastic forces such as genetic drift and the role of historical contingency^{1,2}. However, evolutionary processes can also be studied through experimental evolution, in which populations of organisms are cultivated in laboratory conditions, to observe evolution in real time while controlling and manipulating key parameters such as population sizes, genetic backgrounds, spatial structure, mutation rates and environmental conditions. This approach is particularly powerful for understanding the relative contributions of deterministic and stochastic forces, as replicate populations provide insights into the repeatability of evolutionary outcomes by controlling for initial conditions. Thus, experimental evolution offers opportunities to test hypotheses generated from natural systems in controlled settings and to explore alternative evolutionary trajectories that may not be accessible through the study of historical patterns alone.

Early laboratory evolution experiments pioneered the use of simplified microbial systems to study fundamental questions in evolution^{3–8}. Among the most famous is the *Escherichia coli* long-term evolution experiment (LTEE), initiated by Richard Lenski in 1988, which has tracked the evolution of 12 replicate *E. coli* populations in a simple, constant environment for more than 35 years so far. The LTEE and other evolution experiments have provided important insights into the tempo of genetic evolution⁹, the role of fitness landscapes and epistasis^{10–12}, niche partitioning and eco-evolutionary dynamics^{5,13–15}. These experiments generally rely on relatively small numbers of replicate populations, and have used low-throughput and indirect methods (for example, clonal sequencing^{16,17} and colorimetric^{6,18} or fluorescent markers¹⁹) to measure any evolved genetic and phenotypic changes. Partly because of the limitations of such methods, researchers faced substantial challenges in characterizing the properties of inherently stochastic evolutionary processes, connecting observed genotypic changes to phenotypic outcomes, and establishing clear links between laboratory evolution and natural evolutionary dynamics. These limitations have underscored the critical need for more modern techniques and approaches in experimental evolution.

Over the past two decades, the field of experimental evolution has responded to the need for more modern techniques by undergoing a dramatic transformation driven by advances in high-throughput sequencing and liquid handling^{20,21}. The use of high-throughput sequencing to track genome evolution has now become almost routine in experimental evolution, enabling researchers to track genetic changes over extended time periods and across hundreds or even thousands of replicate populations. Advances in high-throughput library preparation have made it possible to sequence hundreds to thousands of individual clones^{9,22–24} or metagenomic samples^{25,26}, also enabling researchers to quantify the evolution of genetic diversity through time. Furthermore, techniques such as DNA barcoding harness the power of high-throughput sequencing to connect these genotypes to phenotypic outcomes in highly parallelized assays^{27–29}, and new genome-editing methods enable researchers to create millions of genetic variants in parallel^{30–32}. Together, these methods have opened many new avenues of enquiry, such as investigations of the

predictability, repeatability and contingency of evolutionary processes at the genetic level.

In this Review, we examine the technological advances that are reshaping the field of experimental evolution. We begin by overviewing these technologies that enable manipulating and measuring genotypes and phenotypes at scale, specifically barcoding, lineage tracking, high-throughput phenotyping and gene-editing approaches. We then focus on three areas of research for which new tools are having a substantial impact: predicting and potentially controlling evolutionary outcomes, understanding of eco-evolutionary interactions, and bridging the gap between laboratory and natural evolution. As we explore these developments, we discuss how the integration of high-throughput technologies with classical experimental evolution approaches is reshaping our understanding of evolutionary processes and enabling for a more predictive science of evolution.

Manipulating and measuring genotypes and phenotypes at scale

Recent technological advances in high-throughput sequencing, genome editing and phenotyping methods have dramatically expanded our ability to manipulate and measure genetic and phenotypic changes at unprecedented scales, transforming how evolution is studied in the laboratory. These methodological breakthroughs enable researchers to systematically explore genotype–phenotype relationships across thousands of variants simultaneously.

Barcoding, lineage tracking and high-throughput phenotyping

Since its introduction into experimental evolution about a decade ago, DNA barcoding has revolutionized our ability to analyse evolving populations²⁷. This technique involves stably integrating diverse, short (typically 8–30 bp) and random DNA sequences into the genome, permanently marking each lineage with a unique ‘barcode’ identifier (Fig. 1). By amplicon sequencing these barcode regions, researchers can measure the relative frequencies of different lineages. As potential barcode diversity is generally high compared with actual diversity, true barcodes differ by multiple mutations and thus barcodes can be correctly identified even in the face of sequencing errors. Therefore, the resolution of this approach is limited only by sequencing depth and barcode diversity^{33,34}; it is typically possible to detect lineages present at frequencies as low as 10^{-6} .

DNA barcoding has been used to analyse how the dynamics of competing lineages within a population shape long-term evolutionary outcomes. Researchers introduce diverse barcodes at a neutral locus to label lineages within an initially clonal microbial population, and track their frequencies through time as lineages acquire mutations and their frequencies change owing to genetic drift and natural selection^{27,29,35}. Provided barcode diversity is sufficiently high, each lineage initially consists of only a few cells, making it possible to infer the distribution of fitness effects of the mutations that drive short-term evolution³⁶. This approach makes it possible to directly observe evolutionary dynamics in real time and better understand how evolutionary forces shape statistical patterns of genetic diversity and evolutionary trajectories. For example, such work has helped to reveal that beneficial mutations occur at surprisingly high rates in microbial populations, with multiple advantageous variants arising and competing simultaneously^{27,29}. This pervasive clonal interference, rather than sequential selective sweeps, maintains substantial genetic variation within asexual populations even during strong directional selection, blurring the distinction between

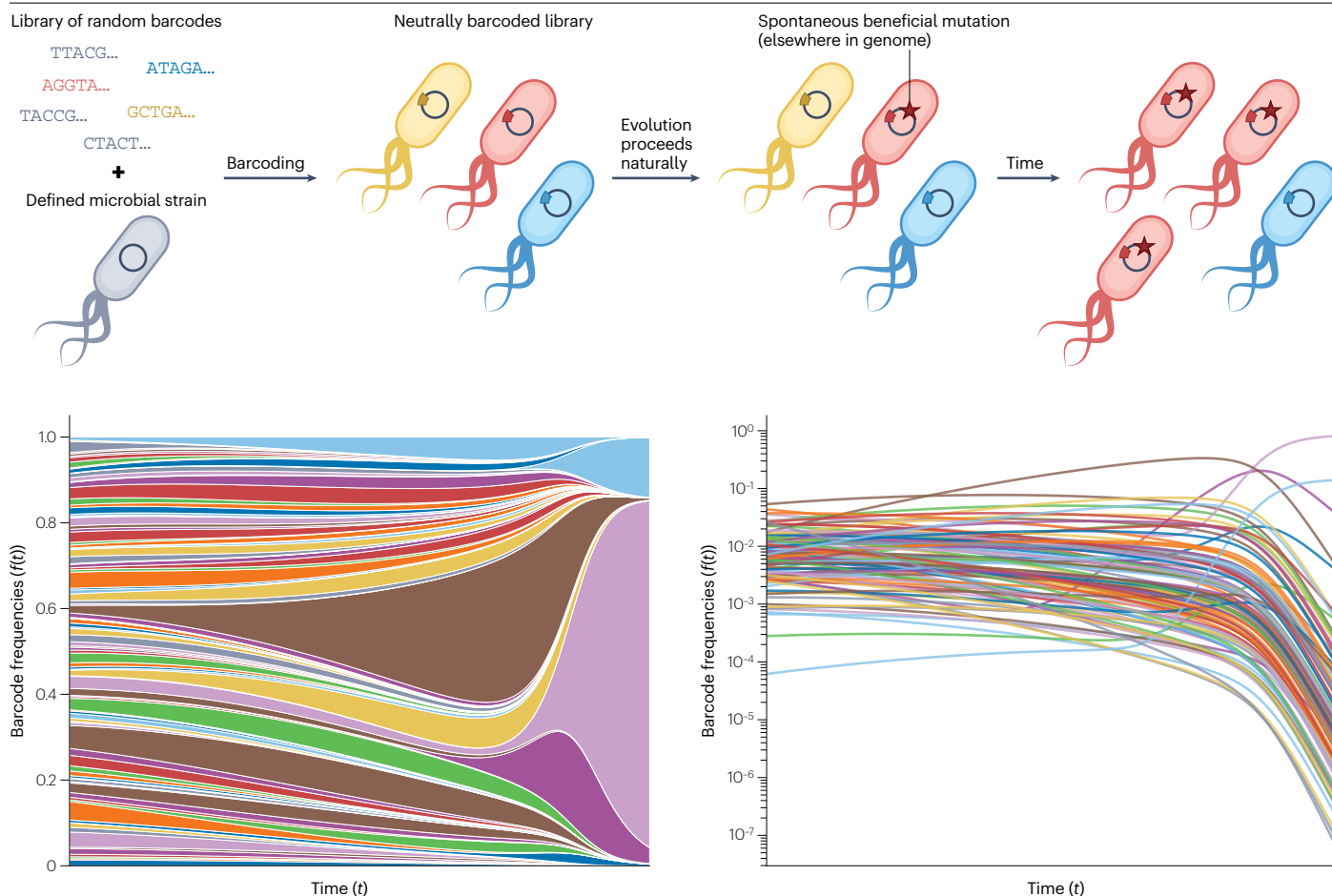


Fig. 1 | Barcoding, lineage tracking and high-throughput phenotyping methods. Molecular barcoding has emerged as a powerful high-resolution tool to track evolutionary dynamics in real time, and to perform high-throughput phenotyping. In a typical experiment, random DNA barcodes are integrated into a neutral genomic location of a defined microbial strain. The relative frequencies of different barcoded strains can be measured by amplicon sequencing.

The population is then allowed to grow in a defined environment, and evolution proceeds naturally – mutations can arise on the background of any barcoded variant, and selection can act on the new genetic diversity. The fitness effects of a given lineage i (s_i) can be directly measured by observing how different barcodes increase or decrease in frequency: $f_i(t + \Delta t) = \frac{f_i(t)e^{s_i\Delta t}}{\sum_j f_j(t)e^{s_j\Delta t}}$.

adaptation from new mutations and adaptation from standing genetic variation.

Barcode tracking experiments face two main limitations. First, selection reduces and eventually eliminates barcode diversity, obscuring future dynamics. Recent work has addressed this challenge by developing methods to efficiently re-barcode populations and periodically restore diversity²⁹. Second, identifying the mutations responsible for fitness changes in specific lineages typically requires labour-intensive single-cell or single-clone sequencing²². Although this limitation can be partially addressed by coupling barcoding with high-throughput genome manipulation methods, such deliberately introduced mutations may not reflect naturally occurring evolutionary changes.

Beyond lineage tracking, DNA barcoding can be used to efficiently measure any phenotype that can be quantified based on relative barcode enrichments. This approach has been valuable in microbial functional genomics for identifying gene function through genome-wide barcoded knockout studies^{28,30,37,38}. For example, researchers have used barcoded knockout approaches to identify genes involved in

stress response, such as heavy metal tolerance and motility³⁰. Bar-coded knockout experiments inspired by such functional genomic screens have recently been used to probe how the distribution of fitness effects changes across evolutionary time^{39,40}, and represent promising approaches in general to measure statistical distributions of evolutionary trajectories and phenotypic effects of mutations. More sophisticated experimental designs enable measurement of other, more complex phenotypes of interest (Box 1). For example, a recent study used barcoded H1N1 influenza virus variants to simultaneously measure neutralization by human sera across numerous related viral strains⁴¹, providing insights into the evolutionary dynamics governing viral escape from antibody-mediated immunity.

Constructing and manipulating genomes

A key challenge in barcoded evolution experiments is connecting observed changes in competitive fitness to their underlying genetic causes and broader phenotypic effects. Even when sequencing identifies mutations in evolved lineages, determining which mutations

Box 1 | Phenotypes important for evolutionary dynamics

We define a phenotype as any measurable property of an organism affected by some combination of genetic, environmental or stochastic causes. Under this definition, the relative reproductive success of a given genotype (that is, its fitness) is considered a phenotype, albeit a special one that directly affects short-term evolutionary dynamics. The high-throughput methods described throughout this Review have dramatically improved our ability to measure fitness — along with other phenotypes — across large numbers of genotypes, enabling us to investigate the statistical patterns that shape evolutionary trajectories.

The distribution of fitness effects of new mutations represents one of the most fundamental statistical properties governing evolution. Experimental measurements across diverse systems have revealed general patterns that shape distributions of fitness effects — such as widespread diminishing returns and increasing costs epistasis — providing evidence for general statistical principles in adaptation despite the idiosyncratic molecular details of specific mutations.

Beyond fitness measurements, it is often unclear which other phenotypes should be measured in an evolving population to gain insight into the underlying evolutionary process. When we have an a priori reason to be interested in specific phenotypes (for example, disease phenotypes, transcriptomes or antibody-binding affinities),

tracking how these phenotypes change across replicate populations and evolutionary time can reveal the forces driving phenotypic evolution. Such information may enable researchers to predict and potentially manipulate phenotypic evolution during future adaptation. For example, by systematically measuring how environmental conditions alter the relationship between specific phenotypes and fitness, experimenters could develop predictive models for how these phenotypes will evolve under new or changing conditions.

Alternatively, we may measure phenotypes because we believe they are important determinants of selection. Quantifying how multiple phenotypes collectively contribute to fitness through statistical modelling approaches might enable us to forecast evolutionary trajectories under novel conditions. When it is unclear which phenotypes contribute to fitness, one study¹⁶⁹ proposed measuring fitness in various environments and using linear decomposition to infer abstracted ‘fitnotypes’ that could predict fitness effects in other environments. However, such predictions face an inherent difficulty: fitness typically emerges from complex, often nonlinear interactions among multiple traits and genetic variants in specific environments. This complexity means that even large phenotypic datasets and sophisticated models may struggle to accurately generalize beyond the immediate neighbourhood of the dataset.

drive phenotypic changes remains difficult owing to the abundance of passenger mutations²⁵. To address this limitation, researchers have developed various high-throughput genome manipulation methods that enable systematic measurement of both competitive fitness effects and, in some cases, specific phenotypes across many engineered genotypes (Fig. 2). Many of these methods were developed to address challenges in functional genomics and have been widely applied to that field^{28,30,38,42}; however, we focus here on how such methods can be applied to questions that arise in experimental evolution. These methods vary in both methodology and scope, ranging from saturating mutagenesis approaches that systematically explore local fitness landscapes to combinatorial methods that examine all possible combinations of selected mutations.

At the genome level, saturating mutagenesis has been implemented through techniques such as barcoded transposon mutagenesis^{28,30}. This approach uses transposons that randomly insert themselves into microbial genomes, disrupting gene expression and creating knockout mutations. With sufficient coverage, every gene in the genome can be disrupted multiple times. A single high-throughput sequencing step links each barcoded transposon to its genomic location, which enables subsequent barcode sequencing to measure the fitness effects of thousands of knockouts in parallel. This technique can provide a crucial bridge between adaptive mutations identified in evolution experiments and their phenotypic consequences by systematically perturbing the same genes that acquire mutations during adaptation. For example, recent work has revealed how knockout effects change during long-term evolution in the LTEE^{39,40}, demonstrating that genes acquiring adaptive mutations often show substantial fitness effects when disrupted, and that these effects change predictably as populations adapt to their environment. Similar studies in *Saccharomyces cerevisiae*⁴³ have shown that the distribution of beneficial and deleterious knockout mutations changes predictably with background fitness,

helping explain the diminishing returns patterns observed across multiple experimental evolution systems. Related approaches include genome-wide barcoded CRISPR interference, which has been used to generate knockdown libraries and titrate gene expression^{44,45}, enabling researchers to correlate the degree of expression change with fitness outcomes and better understand the mechanistic basis of adaptive expression changes. Conversely, genome-wide overexpression libraries can be used to probe the effects of increased expression of any given gene in the genome⁴², often mimicking the effects of promoter mutations observed in evolution experiments. Other methods such as CRISPEY-BAR (Cas9 retron precise parallel editing via homology with barcoding)^{46,47} have been used to introduce specific, natural variants into multiple different *S. cerevisiae* genetic backgrounds. In doing so, this method has revealed widespread background dependence of mutations³¹ and provided direct evidence for how the same adaptive mutations identified in laboratory evolution can have context-dependent phenotypic effects.

Saturating mutagenesis approaches can also be applied at the level of the evolution of individual proteins, through techniques such as deep mutational scanning (DMS)^{32,48,49}. DMS approaches generally aim to quantify not only the competitive fitness effects but also specific molecular phenotypes, such as binding affinity, protein stability or enzymatic activity, for all possible single amino acid changes to a protein using error-prone PCR (or related methods) coupled with pooled assays. DMS approaches have been applied to a wide variety of different proteins, enabling researchers to quantify which sites tolerate mutations and those that might enable fitness gains. These approaches have been used to forecast protein evolution; for example, by using DMS on influenza and SARS-CoV-2 proteins, researchers have been able to predict which evolutionary paths are probably available to the pathogens by uncovering mutations that enhance host cell receptor binding and immune escape^{32,48,49}.

Although approaches that explore the effects of individual mutations generally cannot uncover epistatic effects, combinatorial methods have the potential to uncover pairwise and higher-order interactions between mutations, and thus have been applied to measure the interactions of multiple variants across the genome. These methods

require selecting specific mutations of interest, often based on prior knowledge of potentially adaptive variants. Approaches using methods from standard microbial genetics have generally been limited to at most dozens of different combinations of genetic variants^{10,11}, but newer methods that rely on barcoding combined with newer genetic

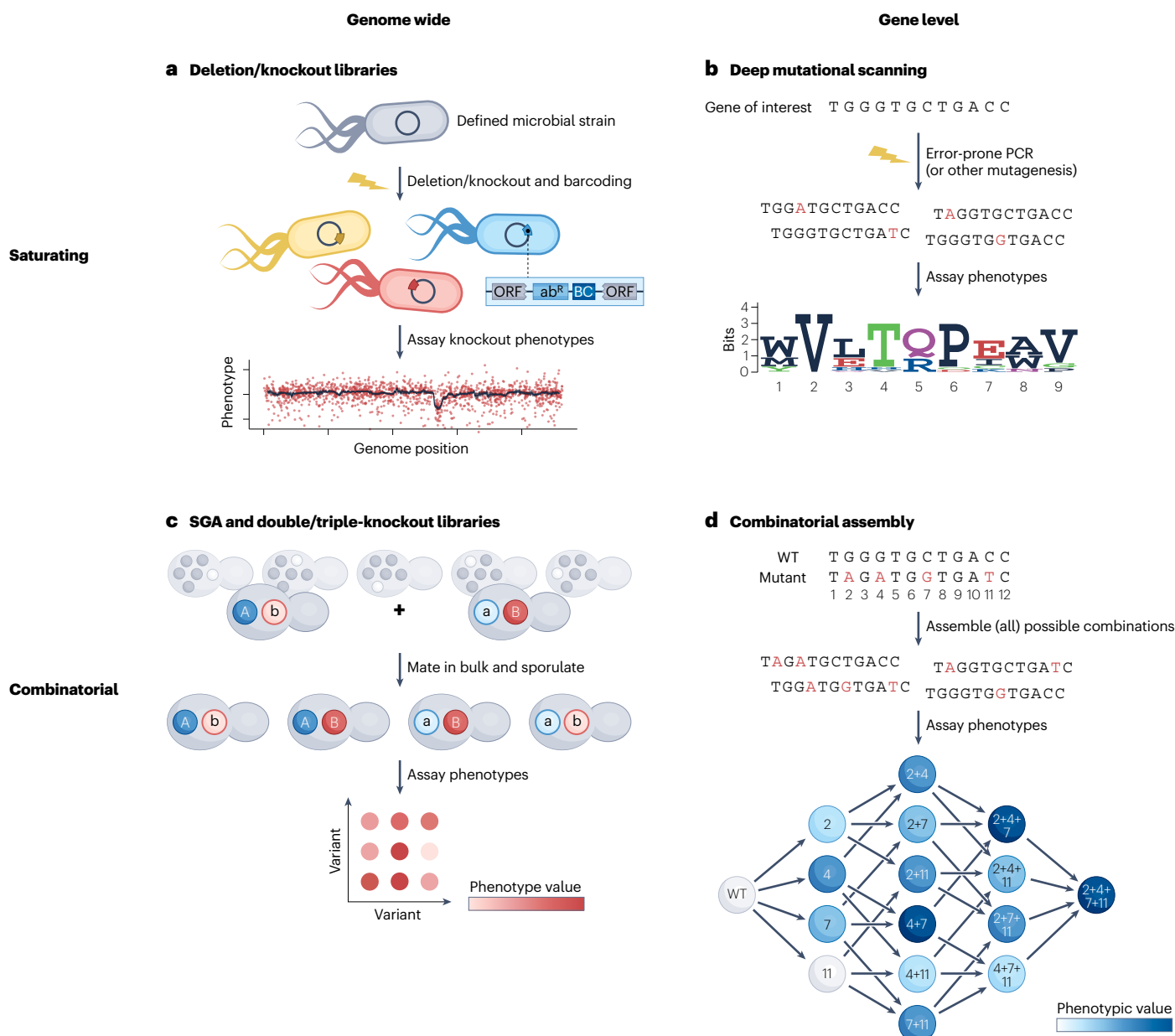


Fig. 2 | Construction and manipulation of genotypes. Several recent methods for genotype manipulation can be characterized in terms of their genomic scope (whole genome versus single gene) and mutation strategy (saturating versus combinatorial). **a**, Genome-wide saturating mutagenesis. Common approaches include barcoded deletion and knockout libraries, often employing transposons with selectable markers that randomly insert into genes to disrupt their function. **b**, Single-gene saturating mutagenesis. This approach employs deep mutational scanning, where a method such as error-prone PCR generates all possible single-nucleotide substitutions in a target gene, followed by high-throughput

phenotype assessment. **c**, Genome-wide combinatorial mutagenesis. Common approaches use synthetic genetic arrays (SGAs) and multiple knockout libraries, particularly in yeast. This approach combines recombinant genetics, mating and selection to create multiple mutants. Modern approaches can incorporate barcodes for high-throughput phenotyping. **d**, Single-gene combinatorial mutagenesis. When comparing evolved and wild-type (WT) genes, all possible intermediate genotypes can be constructed and phenotyped to understand the effects of mutational combinations. ORF, open reading frame.

tools can generate an order of magnitude more combinations. For example, a recent study used a recombining CRISPR-gene drive system in *S. cerevisiae* to construct most of the possible $2^{10} = 1,024$ combinations of 10 mutations that were identified in early stages of adaptation to ultimately investigate how global epistatic patterns emerge from idiosyncratic interactions⁵⁰.

Partly owing to the ease of working with smaller sequences, combinatorial gene-editing methods have been applied more widely at the level of nucleotide sequences and proteins to investigate intra-protein epistatic interactions between residues^{51–56}. As before, a priori information is often needed to choose which genetic variants to include. For example, one study chose to mutate just three residues known to be important in conferring trimethoprim resistance in a key *E. coli* metabolic gene, *folA*, which encodes dihydrofolate reductase⁵⁷. Each of the three residues were mutated such that each site could be any of the 20 amino acids, resulting in nearly $4^3 = 262,144$ variants. This study found a fitness landscape with many separated peaks (indicating a rough landscape), but the highest peaks were all nevertheless found to be accessible through paths that monotonically increase fitness. Another option is to construct all possible evolutionary intermediates between a known wild-type sequence and an evolved sequence^{58–60}. Exploring all intermediates has enabled the elucidation of the evolutionary and epistatic forces that shaped protein evolution and the constraints on evolutionary trajectories. This approach has also proved valuable for understanding how epistatic interactions constrain directed evolution efforts, as demonstrated in studies of industrially relevant proteins such as tryptophan synthase⁶¹.

Predicting and manipulating evolution

A fundamental question in evolutionary biology is the extent to which evolution is repeatable and predictable^{62–64}. The degree and manner of predictability is thought to depend on both the genotype–phenotype–fitness map and the relative strength of various population genetic forces – such as natural selection, genetic drift and mutation – acting on the population^{36,65,66}. Recent studies leveraging high-throughput methods have revealed that evolution typically shows high levels of phenotypic repeatability but relatively lower levels of genetic repeatability. For instance, when replicate populations are evolved under identical conditions, they often achieve similar fitness improvements and converge on similar phenotypic solutions, but the underlying genetic changes can vary substantially across replicates^{12,26,67} (Fig. 3 and Supplementary Information). Such phenotypic repeatability implies a many-to-one relationship between genotypes and phenotypes, and has also been referred to as ‘genetic redundancy’⁶⁸.

The application of high-throughput molecular phenotyping approaches has begun to reveal the mechanistic basis for phenotypic repeatability. These methods have shown how different genetic changes can produce similar effects on phenotypes through various mechanisms. For example, studies combining evolution experiments with transcriptomic profiling have revealed how distinct mutations and evolutionary paths can lead to similar gene expression changes⁶⁹ and similar changes in traits such as cell size⁷⁰. The hierarchy of phenotypic organization has a crucial role in determining the level at which evolutionary outcomes become predictable. Recent work using high-throughput phenotyping has shown that although genetic changes may be highly variable, they often affect a common set of cellular processes or regulatory pathways⁷¹. Similarly, metabolomics approaches have revealed how different genetic changes can lead to similar alterations in metabolic fluxes in experimental systems⁷².

Global patterns of epistasis have emerged as crucial factors driving evolutionary repeatability. Epistasis has long been recognized to have an important role in evolutionary dynamics, but traditional experimental approaches were limited to studying interactions between small numbers of mutations. Experimental evolution helped to shift our understanding of the patterns and role of epistasis by revealing genetic interactions between large numbers of genetic variants across the genome. Specifically, studies tracking adaptation in replicate populations first identified the striking pattern that beneficial mutations yield diminishing returns in more fit backgrounds, whereas deleterious mutations become increasingly costly^{43,67,73}. In contrast to traditional genetic approaches that could generally identify epistasis between specific mutations, experimental evolution with whole-genome sequencing showed how fitness effects depend on the background fitness of the organism, regardless of the specific mutations involved. Such patterns of global epistasis have revealed that whereas epistatic interactions could, in principle, be incredibly high dimensional, they seem generally more low dimensional with the phenotypic effects of mutations being largely explained by background fitness alone (Box 2). This pattern can explain how genetically distinct mutations can nevertheless produce similar phenotypic outcomes, leading to genetic variability despite phenotypic convergence¹². Recent work using high-throughput methods to construct and measure the effects of large numbers of mutations has revealed that these patterns of global epistasis can arise from idiosyncratic epistatic interactions⁵⁰, confirming theoretical predictions⁷⁴.

Eco-evolutionary interactions

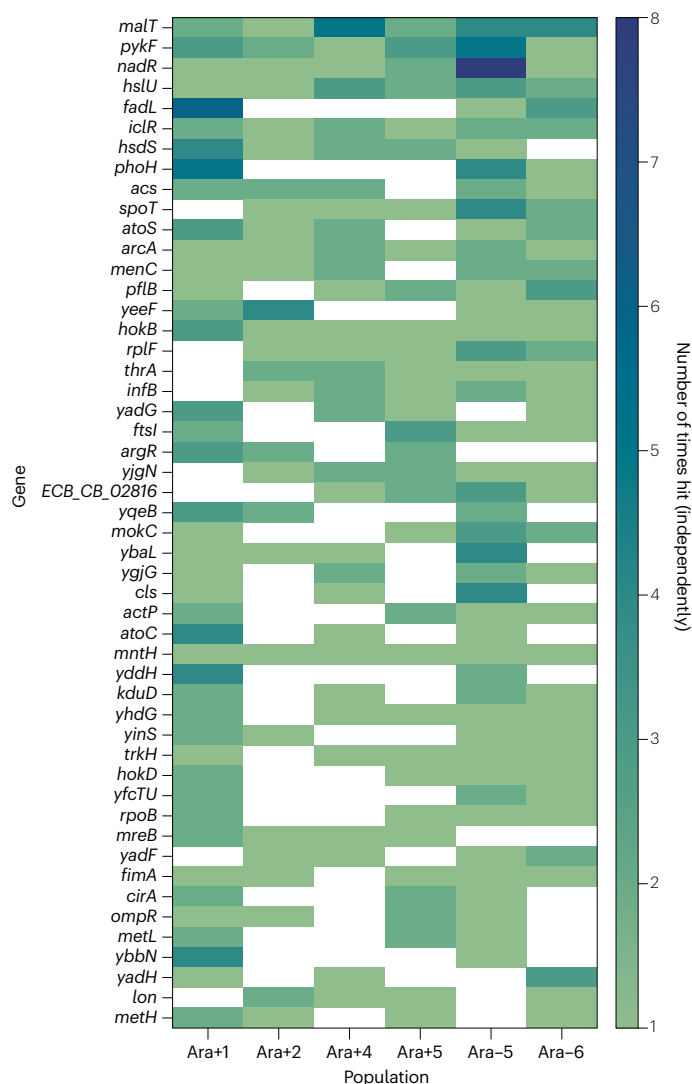
Experimental evolution has repeatedly shown that even in simple, constant environments, microbial populations often develop complex ecological interactions, including the stable coexistence of distinct lineages. High-throughput methods are providing deeper insights into the mechanisms underlying these emergent ecological dynamics and how they shape ongoing evolutionary trajectories.

Characterizing emergent ecological forces

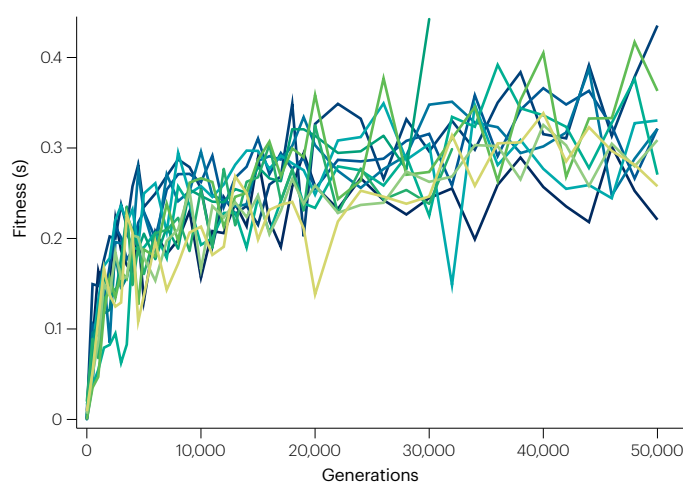
One of the most striking findings in experimental evolution is the spontaneous emergence of negative frequency-dependent selection and stable coexistence of lineages^{5,13,15,26,75–81}. Such ecological diversification has been found to occur with surprising frequency, even in environments specifically designed to minimize ecological complexity⁸² (Fig. 4). These simple ecological communities have been detected across unrelated systems (although they do not arise in all experimental systems⁸³). These lineages maintain their coexistence through various mechanisms, including resource partitioning^{15,84}, spatial niche construction⁷⁶ or cross-feeding^{5,77,85}. Importantly, adaptive evolution continues beyond initial diversification – coexisting lineages coevolve, diverging at genetic, transcriptomic and metabolomic levels^{26,86–88}. This ongoing coevolution can alter the relative frequencies of lineages and, in some cases, lead to the extinction of one lineage, thereby collapsing the ecological system^{26,76,89}. Similar dynamics have been observed in natural populations, including in the microbial communities of the human⁹⁰ and mouse gut⁹¹. Together, these observations have led to the crucial realization that ecological and evolutionary dynamics often occur on similar timescales in microbial communities. This insight has sparked a surge of interest in using experimental evolution to better understand eco-evolutionary dynamics.

High-throughput methods have improved our ability to characterize the nature and evolution of emergent ecological interactions,

a Top 50 most hit genes in the LTEE



b LTEE fitness trajectories



c Changes in LTEE gene expression after 50,000 generations

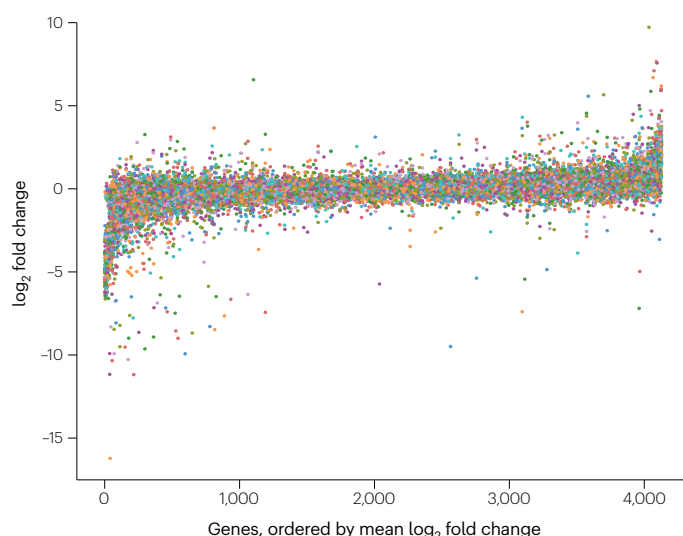


Fig. 3 | Examples of repeatability and predictability in evolution. **a**, Multi-hit genes in non-mutator populations of the *Escherichia coli* long-term evolution experiment (LTEE). Many of the genes that were mutated across multiple populations were also mutated multiple times within populations over time (data obtained from ref. 26; Supplementary Information). **b**, The fitness

trajectories of each population in the *E. coli* LTEE. Fitness increases over time were remarkably similar across all LTEE populations (data obtained from ref. 67; Supplementary Information). **c**, Parallel changes in gene expression across all LTEE populations, from 0 to 50,000 generations (data obtained from ref. 69; Supplementary Information).

particularly frequency-dependent selection and ecological coexistence. For instance, early in the *E. coli* LTEE, it was found that one of the 12 lineages diversified into two stably coexisting lineages; both lineages were maintained in the population for tens of thousands of generations^{13,86}. These lineages were named S and L for their small and large colony sizes, respectively, on certain agar plates; the obvious phenotypic differences were key to uncovering the ecological structure of the population. It was later found that the mechanism of coexistence primarily relied on metabolic niche specialization and glucose-acetate cross-feeding^{14,85,88}. For a time, this ecological diversification was

thought to be a fluke – a population that stumbled upon a rare evolutionary path. However, recent work used densely sampled longitudinal metagenomic sequencing to characterize the genetic changes that have occurred over 60,000 generations of evolutionary time in the *E. coli* LTEE²⁶. Contrary to prior expectations, this study found that ecological diversification into two coexisting lineages occurred in 9 out of the 12 populations and was followed by subsequent coevolution of both lineages. The unbiased and high-throughput nature of the metagenomic sequencing was key to uncovering this widespread emergence of ecological interactions, without depending on observing phenotypic

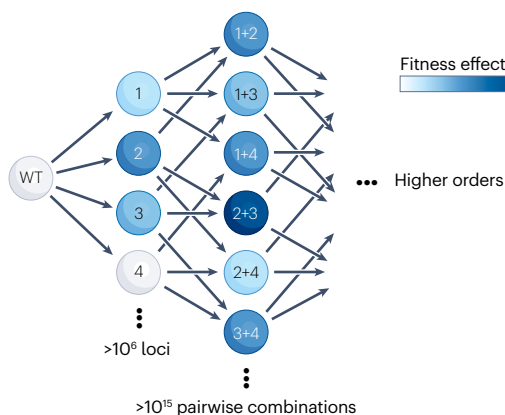
Box 2 | Epistasis and contingency in the predictability and repeatability of evolution

The predictability and repeatability of evolution ultimately depend on the structure of genotype–phenotype–fitness landscapes. Fitness landscapes are inherently complex due to their high dimensionality. In a typical microbial genome, millions of possible single mutations exist around a wild-type (WT) genotype. When considering combinations of mutations, the number of possibilities grows exponentially (Box 2 figure, left panel). Whereas earlier work focused primarily on mapping fitness landscapes for individual proteins or small genetic systems^{10,11,56}, new high-throughput approaches enable the exploration of genome-wide landscapes. These studies have revealed several key patterns in the architecture of fitness landscapes, suggesting that low-dimensional representations of fitness landscapes are often possible owing to global patterns of epistasis and many-to-one genotype–phenotype relationships. For instance, many studies have documented widespread ‘global’ epistasis, where the effect of a mutation depends on a higher-level property of genetic background in which it occurs^{11,12,43}. This global epistasis often takes a specific form, where the effect of a mutation is negatively correlated with the fitness of the genetic background (Box 2 figure, right panel)⁷⁴. Such diminishing returns and increasing costs epistasis helps explain how different genetic changes can lead to similar phenotypic outcomes, contributing to the observed

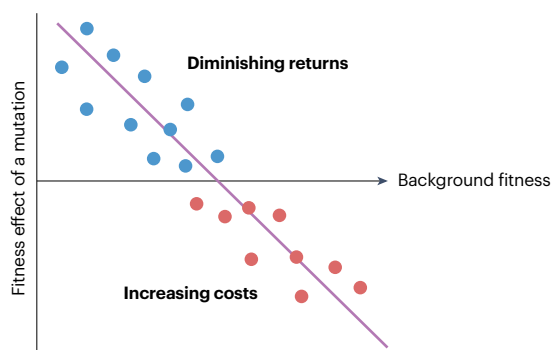
patterns of high phenotypic but low genetic repeatability in evolution experiments^{9,26,67}. These simple patterns may emerge from the combined effects of many higher-order, idiosyncratic epistatic interactions^{50,74}.

Another crucial feature that emerges from widespread epistasis is the prevalence of contingency, such that the availability of beneficial mutations depends strongly on previous evolutionary history. For example, studies combining systematic genetic perturbations with molecular phenotyping have shown how proteins and organisms can evolve new functions through specific sequences of mutations, with each step requiring previous changes to be accessible⁷⁵. Understanding these landscape features is essential for predicting evolutionary outcomes and potentially for steering evolution in desired directions. For instance, knowledge of landscape architecture can help identify genetic changes that might facilitate or constrain future adaptation, as well as suggest intervention strategies to prevent the evolution of antibiotic resistance or slow cancer progression. As our ability to map and analyse these landscapes continues to improve, we may develop increasingly sophisticated approaches for predicting and manipulating evolutionary trajectories in both laboratory and natural settings.

Full fitness landscapes are very high dimensional



Lower-dimensional representations through global epistasis



differences. High-throughput measurements have also recently been applied to better understand the nature of the eco-evolutionary forces in the LTEE. Barcoded transposon mutagenesis was recently applied to *S. aureus* ecotypes immediately after they diversified, which revealed a fitness landscape dependent on community composition and predictive of evolutionary outcomes³⁹.

Artificial and assembled communities

A complementary approach to understanding ecological interactions during evolution comes from the emerging field of experimental ecology. This approach, often led by the same groups that study experimental evolution and employing similar high-throughput methods, provides crucial insights into the principles governing community assembly and stability, which are essential for understanding evolution

in complex natural systems. By constructing artificial microbial communities with defined compositions, researchers can systematically test hypotheses about ecological interactions that would be difficult to isolate in natural settings^{92,93}.

Ecosystems are sometimes thought to be intractably complex, depending on a multitude of idiosyncratic interactions that challenge our ability to predict and understand these systems. However, some recent findings have suggested that community structure is governed by a type of emergent simplicity. For example, high-throughput metagenomic approaches have revealed that the natural microbiomes of bromeliads vary substantially between plants on the taxonomic level, but are surprisingly consistent on a functional level^{94,95}. This observation suggests that community assembly may be predictable, provided that we look at the right level of organization.

With the help of high-throughput metagenomics, such predictability on the functional (but not taxonomic) level has been recapitulated in experimental microbial communities, which can be explained with consumer–resource models^{93,96}. Simple, quantitative rules have been found to be surprisingly powerful in predicting the outcomes of microbial community assembly experiments^{97–100}. For example, the outcomes of community assembly experiments have also been found to be largely predictable by applying quantitative rules to small numbers of initial measurements⁹². Such ‘assembly rules’ may again be explained by consumer–resource dynamics¹⁰¹. If these patterns prove broadly applicable across different ecosystems, they may provide the insights needed to predict and control ecological dynamics with relatively limited data.

The integration of these ecological insights with evolutionary approaches represents a promising frontier for understanding eco-evolutionary dynamics. Although we have begun to uncover the rules governing community assembly and function, how these principles shape and are shaped by evolutionary processes remains an open question. Recent pioneering work has begun evolving simple artificial communities to investigate these dynamics, which has led to preliminary evidence for quantitative eco-evolutionary rules that parallel those seen in community assembly^{102,103}. These studies suggest that the predictable patterns emerging from ecological interactions may extend to evolutionary trajectories as well.

As high-throughput molecular methods continue to advance, the integration of experimental ecology and evolution offers a powerful

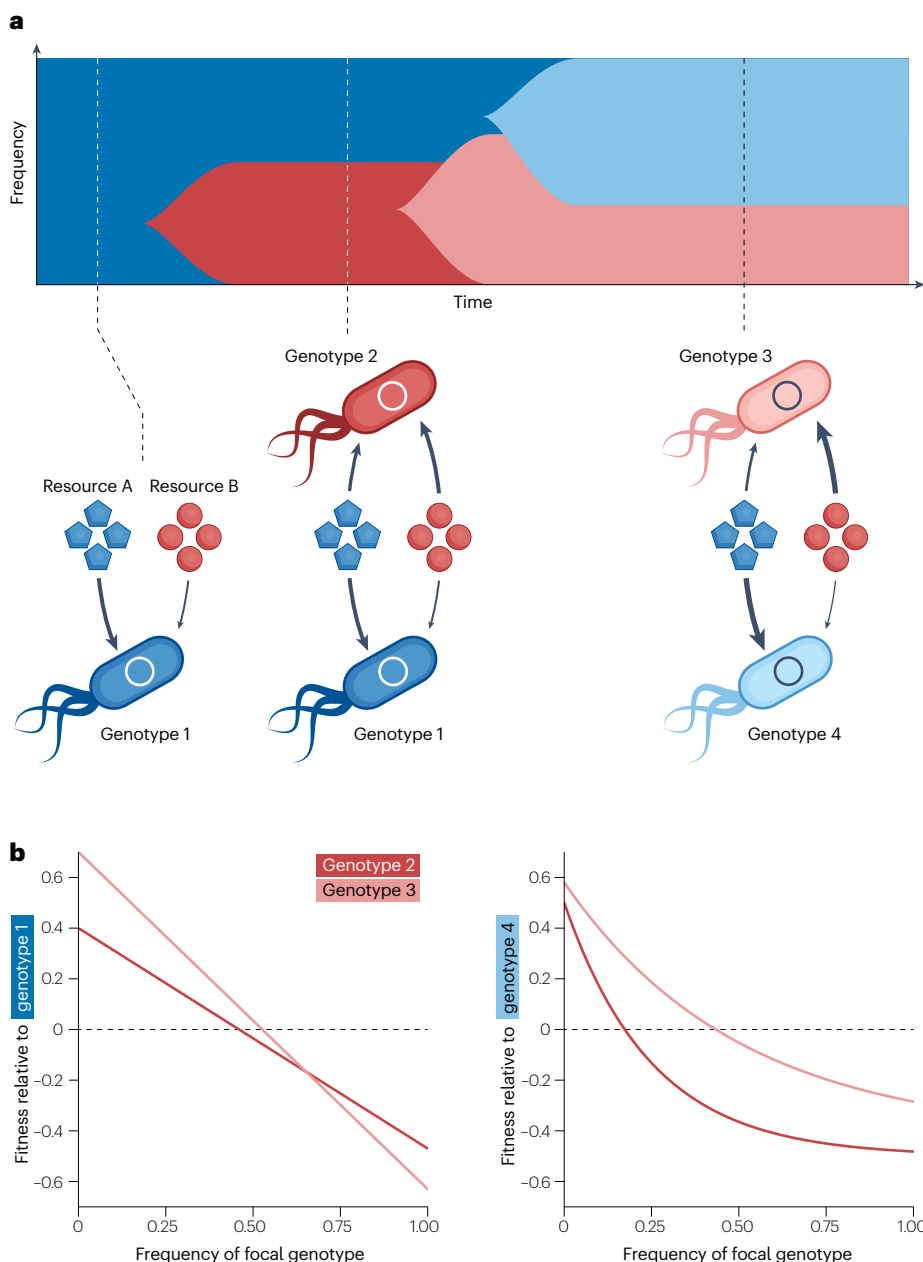


Fig. 4 | Evolution of frequency-dependent interactions. Multiple evolution experiments have shown how initially uniform populations develop ecological specialization. **a**, These experiments typically begin with a single genotype growing in a constant environment, perhaps with multiple substitutable resources. Mutations can generate new genotypes that specialize in using one resource more efficiently, at the cost of reduced growth on alternative resources. Subsequent mutations further modify resource consumption patterns within each lineage. **b**, These evolutionary changes lead to frequency-dependent fitness effects, where the success of a genotype depends on the relative abundance of other genotypes in the population.

framework for addressing fundamental questions about the predictability of evolution in complex ecological contexts. This approach may help resolve how the emergent simplicity observed in ecological systems influences the repeatability and contingency of evolution, and how evolutionary processes in turn reshape ecological interactions. Developing a quantitatively predictive understanding of evolution in multispecies communities represents a substantial challenge that will shape the field in coming years, potentially helping to bridge the gap between simplified laboratory evolution and the complexity of natural systems.

Laboratory versus natural evolution

Laboratory evolution experiments serve as powerful model systems for understanding natural evolution, but it often remains unclear which aspects of experimental evolution can be generalized to natural systems. In this section, we explore how high-throughput sequencing and phenotyping methods offer new insights into the similarities and differences between evolutionary processes in controlled laboratory settings and in nature. We then examine two complementary trends that are bridging the gap between laboratory and natural evolutionary studies: the development of laboratory environments that more closely mimic complex natural systems, and the application of experimental evolution techniques to track and analyse evolution in natural settings. These emerging approaches demonstrate how the integration of laboratory and natural studies is enhancing our ability to understand and potentially predict evolutionary outcomes in complex biological systems.

Key differences between laboratory and natural evolution

Laboratory evolution experiments are intentionally designed as simplified models of natural populations. These experimental systems often feature low interspecies diversity, minimal spatial structure, absence of recombination and simplified environments^{83,104}. Despite these stark differences, experimental evolution has uncovered evolutionary phenomena that prove to be important in natural settings. One such example is clonal interference¹⁰⁵, in which multiple beneficial mutations arise simultaneously in large asexual populations and compete for fixation^{25,106–109}. This phenomenon can substantially alter evolutionary trajectories by, for instance, hindering the fixation of moderately beneficial alleles³⁶. Initially described in detail in laboratory populations, clonal interference has since been recognized as crucial in the evolution of natural asexual populations, including within-host tuberculosis populations^{110,111} and global influenza populations¹¹². Similarly, ecological diversification through cross-feeding or resource partitioning – extensively studied in experimental settings^{15,78,85} – has been observed in some natural populations⁹¹.

As the evolution of natural populations in real time has become more deeply studied, other studies have revealed key differences between laboratory and natural evolution. For example, although some natural microbial populations evolve asexually (often over short timescales), it has become increasingly clear that horizontal gene transfer has a substantial role in natural microbial evolution^{113–116}, which is scarcely studied in laboratory evolution. Horizontal gene transfer enables the recombination of genetic variants across backgrounds (within and between species), limiting the effects of clonal interference and enabling deleterious mutations to be purged more efficiently^{117,118}. The rates and lengths of transferred genetic fragments can vary widely across strains¹¹⁹. Overall, horizontal gene transfer may be a more important force generating diversity than spontaneous mutations in many natural systems¹¹⁶. Additionally, although epistasis has often been

shown to be widespread in experimental populations, the importance of epistasis in natural populations with pervasive recombination is still largely unclear.

The mode of evolution in natural systems also seems to deviate at times from the expectations of experimental evolution. More specifically, purifying selection and neutral forces seem to be stronger in natural versus experimental systems, as indicated by comparing dN/dS ratios¹²⁰ and effective population sizes¹²¹. Although it is often unclear why neutral forces are generally stronger in natural (versus experimental) systems, possible reasons include occasional severe bottlenecks caused by strongly fluctuating environments or predation, or large variability in offspring numbers. The nature of ecological interactions may also systematically differ in natural microbial populations, especially as the important role of phage-mediated interactions has become apparent in recent years⁹⁰.

Towards more complex laboratory systems

Recent efforts have focused on studying evolutionary dynamics in more complex laboratory systems, which retain a degree of experimental control while also introducing selected complexities of natural systems. Two notable examples of this approach are genetically tractable mouse models for cancer evolution and in vivo studies of gut microbial communities.

Genetically tractable mouse models have emerged as a powerful system to study cancer evolution in vivo¹²². Such systems have enabled researchers to probe previously inaccessible fundamental evolutionary questions concerning the functional importance of mutation order and how epistatic interactions between mutations shape evolutionary trajectories in cancer. Although the sequence of genetic changes in cancer evolution has been documented in many cases^{123–125}, the evolutionary significance and fitness consequences of such mutations remained speculative. Technologies such as Cre-loxP¹²⁶, transposon mutagenesis¹²⁷ and CRISPR systems^{128,129} enable researchers to induce targeted genetic changes in mice, connecting genetic alterations with phenotypic and evolutionary outcomes. Low-throughput approaches have been used to quantify the fitness effects of cancer-driver mutations¹³⁰ and uncover epistatic effects¹³¹. The development of DNA barcoding in cancer models has substantially increased the power and throughput of experiments characterizing evolutionary dynamics and genotype–phenotype maps in various cancer models^{132–134}. For instance, Tuba-seq (tumour barcoding with ultradeep barcode sequencing) leverages CRISPR–Cas9 to mediate the inactivation of different tumour suppressors in parallel in genetically engineered mouse models of lung adenocarcinoma^{134,135}. Many replicate tumours are formed across several mice by each oncogenic genetic change and, by deep sequencing the barcodes across all tumours, researchers can determine the relative size of the tumours and associate each with genotype. This technology has been used to determine the effects of different nucleotide changes within the KRAS gene¹³⁵, the effects of diverse oncogenic mutations across the genome^{136,137}, and has revealed widespread epistasis and context-dependence among cancer-driver mutations^{138,139}.

The resident microbial communities of animal guts have also emerged as a promising in vivo system to study evolution in real time. A number of model host animals have been used for this purpose, including *Drosophila melanogaster*¹⁴⁰, zebrafish (*Danio rerio*)^{141,142} and *Caenorhabditis elegans*¹⁴³. Mice have been an especially common system to study intra-host evolution, especially owing to the large population of microbes supported in the gut¹⁴⁴ and the similarity to other mammalian guts, including those of humans. Germ-free mice

are readily produced, and the microbial community of the mouse gut can be carefully manipulated. Studies of gut microbial evolution typically involve colonizing the gut with a single species (for example, *E. coli*¹⁴⁵) or a defined collection of a small number of species¹⁴⁶. Such experiments have been crucial in better understanding how the tempo and mode of evolution varies by host environment.

Most experiments track lineages using low-resolution methods, such as the use of two otherwise neutral strains labelled with different fluorescent markers^{147,148}, or through clonal sequencing^{116,149}. Such studies have revealed that adaptive evolution in the mouse gut is often rapid, with complex and shifting selective forces^{145,147,150}. Recently, the application of DNA barcoding to gut communities has enabled high-resolution lineage tracking by deep sequencing the barcode region in the microbial fraction of stool samples^{151–153}. In one study, barcoding was used to measure the distribution of fitness effects of spontaneous mutations in gut microbial populations, revealing differences across strains and environments¹⁵⁴. Another study found reproducible mutations in genes associated with motility and metabolism, and quantified the high prevalence of homogenizing migration of gut microbes between co-housed mice¹⁵³. These studies demonstrate the broad potential of high-throughput methods for understanding evolution in gut microbial communities.

Tracking evolution in natural systems

For decades, a fundamental question in evolutionary biology has been whether the principles derived from experimental evolution accurately reflect the dynamics in complex natural environments. Until recently, this question remained largely theoretical, as the tools to track evolution in natural systems with sufficient resolution were unavailable. However, technological advances originally developed for experimental evolution have now enabled researchers to observe and analyse evolutionary dynamics in natural populations in real time, creating opportunities to test key theoretical predictions about selection, adaptation, and genetic or ecological diversity.

One area where these techniques have been particularly impactful is in studying viral evolution within individual hosts and across populations. For example, deep sequencing of viral populations has enabled researchers to track the emergence and spread of new variants of influenza and SARS-CoV-2 (refs. 155–157). These studies have revealed the complex interplay between immune pressure, antiviral treatments and viral evolution, thus providing crucial insights for vaccine development and pandemic preparedness. Similarly, longitudinal sequencing of HIV populations within infected individuals has illuminated the evolutionary arms race between the virus and the host immune system, revealing patterns of adaptation and escape that occur over the course of infection^{158,159}.

In microbial ecology, metagenomic time-series analyses of the human gut microbiome have transformed our understanding of within-host evolution. Before these studies, the gut was often viewed as a relatively stable community with limited ongoing evolution. However, longitudinal analyses have revealed rapid within-host adaptation^{90,119–121,160,161}, showing rapid evolutionary rates comparable to those observed in laboratory systems despite vastly different ecological contexts. Such approaches have shown how short-term evolution can shift the ecological structure of gut microbial communities¹⁶¹ and quantified the importance of horizontal gene transfer in the evolutionary dynamics across species¹¹⁹. However, experimental results must be interpreted carefully: when microbes are introduced into a new host environment, rapid adaptation may represent a transient adaptation phase that differs

from the evolutionary dynamics occurring when microbes have adapted to their host environment over longer evolutionary timescales.

Industrial and agricultural settings have also emerged as valuable ‘natural experiments’ for studying evolution by combining the control of laboratory settings with the complexity of more natural environments. For instance, metagenomic sequencing has been used in combination with clonal sequencing and novel statistical methods to track lineage dynamics at high resolution in yeast populations in bioethanol refineries¹⁶². Similarly, studies of yeast evolution in beer fermentation tanks have revealed the evolutionary dynamics of populations on both a genomic and proteomic level, with implications for improving beer production^{163,164}. In a field experiment set in an orchard, researchers tracked the evolution of genotypes and phenotypes with diverse *D. melanogaster* populations, revealing the pervasiveness of fluctuating selection and environmental tracking^{165,166}. Subsequent experiments by the same group revealed that selection during population expansion under laboratory conditions can identify alleles involved in adaptation to natural environmental fluctuations¹⁶⁷. This finding suggested that shared selective pressures (probably related to population density changes) operate across environments and that fluctuating selection patterns in *D. melanogaster* may be driven by fundamental life-history trade-offs emerging from boom–bust population dynamics.

These approaches share methodological foundations with experimental evolution but face additional challenges, including uncontrolled variables, sampling limitations and the difficulty of establishing causality. However, they provide crucial tests of laboratory-derived principles in natural contexts. By determining which evolutionary patterns are robust across settings and which are context dependent, these studies help address the central question of when and how laboratory findings can inform our understanding of natural evolutionary processes. As these methods continue to mature, the integration of experimental evolution with natural evolution studies promises to yield a more comprehensive understanding of evolutionary dynamics across scales of biological complexity.

Conclusions and perspectives

The field of experimental evolution has undergone a remarkable transformation in recent years, driven by technological advances that have dramatically enhanced our ability to manipulate genotypes and measure phenotypes. These methodological breakthroughs have not only enabled us to revisit long-standing evolutionary questions with unprecedented precision but have also opened entirely new avenues of enquiry.

Looking ahead, we anticipate continued methodological advancements in several key areas. Genome editing and manipulation techniques, including CRISPR-based methods and high-throughput cloning approaches, are likely to see further refinement. These improvements will allow for the creation of large libraries of genetic variants, enabling more comprehensive explorations of mutational effects and epistatic interactions. Simultaneously, we expect continued development in high-throughput measurements of molecular phenotypes, such as advanced single-cell sequencing technologies and improved metabolomics approaches. The synergy between these two areas of advancement – genome manipulation and phenotype measurement – will provide novel opportunities to directly connect genetic changes to their phenotypic consequences and to systematically investigate genotype–phenotype maps at a previously inaccessible scale.

These technological developments will enable systematic investigation of several fundamental questions. First, combining high-throughput

Review article

Glossary

Background fitness

The overall fitness of a genetic background upon which additional mutations occur; it serves as a baseline for evaluating the fitness effects of new mutations.

Clonal interference

The competition between multiple beneficial mutations that arise in competing lineages within large asexual populations, ultimately slowing fixation rates. This phenomenon can substantially alter evolutionary trajectories by hindering the fixation of moderately beneficial alleles in favour of strongly beneficial ones.

Clonal microbial population

A population derived from a single progenitor cell, where all individuals initially have identical genotypes.

Cross-feeding

An ecological interaction where the metabolic by-products of one organism serve as resources for another organism.

Deep mutational scanning

A high-throughput technique that systematically quantifies the effects of single (and sometimes multiple) amino acid substitutions in a protein. This approach enables researchers to map protein fitness landscapes at high resolution and predict evolutionary trajectories.

Diminishing returns

A pattern of epistasis where beneficial mutations yield progressively smaller fitness gains when introduced into more fit genetic backgrounds. This phenomenon has been widely observed across experimental evolution systems and helps explain convergent fitness trajectories despite genetic divergence.

Directional selection

A form of natural selection that consistently favours traits at one extreme of the phenotypic distribution, driving evolutionary change in a specific direction.

Distribution of fitness effects

The statistical distribution describing the probability that a new mutation has a given fitness consequence. This fundamental property shapes evolutionary dynamics and can change depending on environmental conditions and genetic background.

dN/dS ratios

The ratio of non-synonymous substitutions per non-synonymous site (dN) to synonymous substitutions per synonymous site (dS), providing a measure of selection pressure on protein-coding genes. Values below one typically indicate purifying selection, whereas values above one suggest positive selection.

Effective population sizes

A measure of the strength of genetic drift in a population, often used to summarize genetic diversity in neutrally evolving populations. It is often smaller than the census population size owing to various factors including reproductive variance and population bottlenecks.

Epistasis

The phenomenon where the phenotypic effect of a mutation depends on the presence or absence of other genetic variants. Epistatic interactions can substantially shape evolutionary trajectories by making certain mutational pathways more accessible than others.

Fitness landscapes

A conceptual framework representing the relationship between genotype and fitness, where height on the landscape corresponds to fitness. Valleys represent low-fitness genotypes, peaks represent high-fitness genotypes and the topography influences evolutionary trajectories.

Fixation

The process by which a genetic variant increases in frequency until it is present in all individuals in a population, replacing all alternative variants (at that genetic locus).

Global epistasis

A pattern where the fitness effect of a mutation correlates primarily with the overall fitness of the genetic background, rather than with specific mutations in that background. This pattern enables the projection of complex epistatic interactions onto a simpler low-dimensional space.

Historical contingency

The dependency of future evolutionary trajectories on prior evolutionary history. This concept emphasizes how chance events and the specific sequence of mutations can substantially influence subsequent evolutionary possibilities.

Idiosyncratic interactions

Specific epistatic interactions between particular mutations that cannot be predicted from general patterns. These interactions form the underlying basis from which broader patterns (such as global epistasis) emerge.

Negative frequency-dependent selection

A form of selection where the fitness of a phenotype decreases as its frequency in the population increases. This selective pattern can maintain genetic diversity and lead to stable polymorphisms in evolving populations.

Niche partitioning

The process by which competing species or subpopulations use different resources or adopt different ecological strategies, reducing direct competition and enabling coexistence.

Purifying selection

A form of natural selection that removes deleterious mutations from a population, maintaining functional genetic sequences.

Resource partitioning

An ecological process where different lineages evolve to specialize on distinct components of available resources, reducing competition and enabling stable coexistence.

Selective sweeps

The process by which beneficial mutations increase in frequency and eventually reach fixation in a population, often eliminating genetic variation at linked sites.

Spatial niche construction

The process by which organisms modify their physical environment, creating or altering spatial niches that can be exploited by themselves or other organisms.

Standing genetic variation

The genetic diversity present within a population prior to a selective event, as opposed to variation arising from new mutations. This existing variation can provide an immediate substrate for adaptation to new environments.

phenotyping with experimental evolution across multiple environments will enable the development of predictive models of adaptation that generalize beyond specific conditions. Second, integrating evolutionary and

ecological measurements in microbial communities has the potential to reveal the rules governing the evolutionary emergence and stability of ecological interactions. Recent work demonstrating that microbial

community evolution may follow simple quantitative rules opens the door to potentially enabling prediction and control of complex natural communities^{102,103}. Third, we foresee stronger connections between observations of evolution in nature and the laboratory. Systematic studies of diversity in natural microbial communities, such as on cyanobacterial diversity and genome structure¹⁶⁸, have revealed patterns that often seem at odds with what we observe in laboratory evolution. By applying high-throughput phenotyping and genotyping methods to natural populations, and by designing laboratory experiments that more closely mimic the complexity of natural environments, we may finally be able to reconcile these seemingly discordant observations.

The rise of high-throughput technologies is reshaping our understanding of evolutionary processes. A key challenge for evolutionary biology will be to integrate these large-scale empirical datasets with theoretical models to develop a more quantitative and predictive framework of evolution. Such a framework should explain both observed patterns of biological diversity and accurately forecast evolutionary trajectories in controlled experiments. Through this effort, we may not only deepen our understanding of life's fundamental processes but also develop new tools to address pressing societal challenges, from managing antibiotic resistance to engineering microbial communities for sustainable biotechnology.

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Author contributions

The authors contributed equally to all aspects of the article.

Competing interests

The authors declare no competing interests.

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