Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Genetics & Development

Challenges and potential solutions for studying the genetic and phenotypic architecture of adaptation in microbes

Leandra Brettner[1], Wei-Chin Ho[1], Kara Schmidlin[1], Sam Apodaca[1], Rachel Eder[1,2] and Kerry Geiler-Samerotte[1,2]

All organisms are defined by the makeup of their DNA. Over billions of years, the structure and information contained in that DNA, often referred to as genetic architecture, have been honed by a multitude of evolutionary processes. Mutations that cause genetic elements to change in a way that results in beneficial phenotypic change are more likely to survive and propagate through the population in a process known as adaptation. Recent work reveals that the genetic targets of adaptation are varied and can change with genetic background. Further, seemingly similar adaptive mutations, even within the same gene, can have diverse and unpredictable effects on phenotype. These challenges represent major obstacles in predicting adaptation and evolution. In this review, we cover these concepts in detail and identify three emerging synergistic solutions: higher-throughput evolution experiments combined with updated genotype-phenotype mapping strategies and physiological models. Our review largely focuses on recent literature in yeast, and the field seems to be on the cusp of a new era with regard to studying the predictability of evolution.

Addresses
[1] Center for Mechanisms of Evolution, Biodesign Institute, Arizona State University, USA
[2] School of Life Sciences, Arizona State University, USA

Corresponding author: Kerry Geiler-Samerotte (kerry.samerotte@asu.edu)
Twitter account: L. Brettner (@LeandraBrettner), W.-C. Ho (@wchoEvo), K. Schmidlin (@KaraSchmidlin), S. Apodaca (@SamApodaca_), K. Geiler-Samerotte (@KSamerotte)

Current Opinion in Genetics & Development 2022, **75**:101951

This review comes from a themed issue on **Evolutionary Genetics**

Edited by **Christian Landry** and **Gianni Liti**

For complete overview of the section, please refer to the article collection, "Evolutionary Genetics"

Available online 4th July 2022

https://doi.org/10.1016/j.gde.2022.101951

Introduction

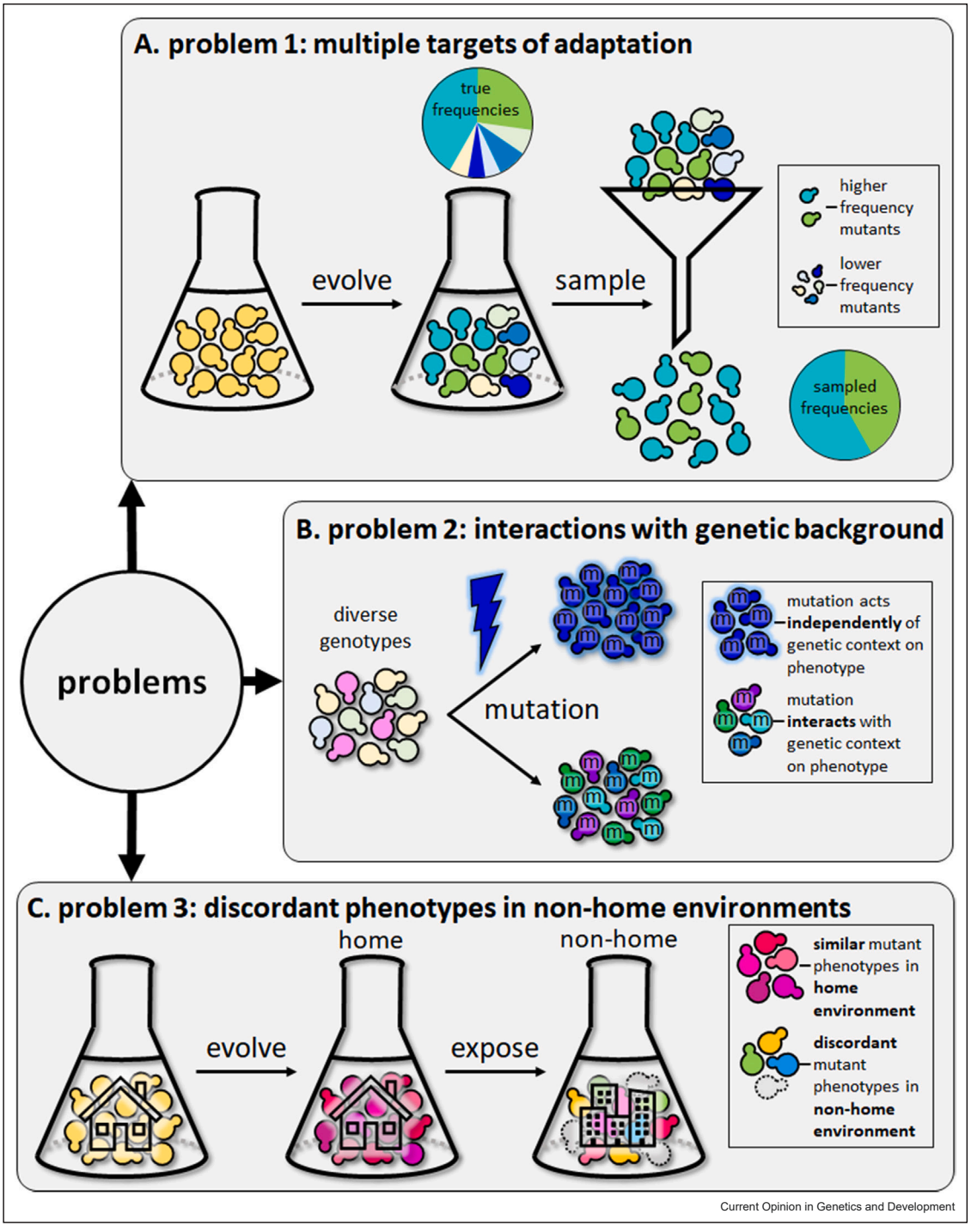
Evolution, as a dynamic process, has proven to be hard to predict. One way populations evolve is through adaptation, or the acquisition of traits that provide an increase in fitness in a given environment. Adaptation is an inherently competitive process, as adaptive mutations in asexually reproducing microbes must survive challenges such as clonal interference, genetic drift, deleterious hitchhiking, etc. to overcome their peers. Gaining a better understanding of the genetic and phenotypic changes that allow organisms to adapt to changing environments, and the broader effects of these changes, is critical to improving the predictability of evolution. In the simplest situation, the possible adaptive genetic targets in a given context are limited or all share similar characteristics, e.g. they all fall into the same handful of genes or molecular pathways that affect similar phenotypes, and the routes to adaptation are easy to predict. This is sometimes true, for example, in scenarios with incredibly strong selective pressures such as adaptation to an essential gene knockout, to nearly fatal antibiotic drug concentrations, etc. [1–3]. However, this has not borne out to be the case in the majority of situations, those in which selection is non-lethal, and the development of reliable tools or rules to predict adaptation have eluded us.

Here, we discuss challenges and recent advances in understanding the genetic architecture of adaptation, many of which also apply more generally to understanding genotype-phenotype mapping [4–6]. Many advances in this field were made possible due to the use of higher-throughput technologies that were developed in or have been applied to the model organism budding yeast, *S. cerevisiae*. These higher-throughput studies have revealed three problems (Figure 1) that impede evolutionary prediction: 1) the genetic targets of adaptation are often more complex than previously realized, 2) the genetic targets of adaptation change with genetic background, and 3) seemingly similar adaptive mutants have diverse and unpredictable effects on phenotype. We discuss these three problems in the first half of this review, then we suggest possible solutions that may yet allow evolutionary predictions in the future.

Problem 1: the genetic targets of adaptation can be numerous and varied

Some studies suggest that only a handful of genes represent potential targets of adaptation to a given stressor.

Figure 1



The problems associated with predicting adaptation. **(a)** Problem 1: the genetic targets of adaptation can be numerous and varied. Traditional methods of isolating adaptive mutants from experimental evolutions were often too low-throughput to catch lower frequency adaptive lineages, leading to the hypothesis that selection only targeted a handful of genes in a given context. **(b)** Problem 2: the genetic basis of adaptation changes with genetic background. Mutations rarely affect phenotype in a way that is independent of the existing genetic background (dark blue yeasts). Instead, often epistasis leads to unpredictable phenotypes when a new mutation interacts with its genetic context (multicolored yeasts). **(c)** Problem 3: seemingly similar adaptive mutants have diverse and unpredictable effects on phenotype. Mutants evolved in a given condition, the 'home' environment, have a similar phenotype: increased fitness in the home environment. One might expect them to have an equally uniform response to a new, 'non-home', environment. However, the pleiotropic effects of mutations often make the response to novel conditions discordant and unpredictable.

In cases where this is true, it yields simpler predictions about how adaptation will proceed. For example, it is widely accepted in *Saccharomyces cerevisiae* and *Plasmodium falciparum* that resistance to the drug pyrimethamine is acquired by ordered, sequential fixation of mutations in *DHFR* or *pfdhfr*, respectively [7–9]. And indeed, *P. falciparum* clinical samples follow these predictions as four-point mutations (N51I, C59R, S108N and I164L) in *pfdhfr* tend to result in failed treatment [10–12]. Unfortunately, this one drug-one gene model is the exception rather than the rule. One study investigating the genetic basis of azole resistance evolved six yeast populations in a clinically relevant concentration of fluconazole and identified four genes, *CDR1*, *CDR2*, *MDR1* and *ERG11* as possible targets of adaptation [13]. Further complicating matters, within a single gene, *ERG11*, not all mutations observed in the clinic provide resistance [14]. Furthermore, more recent studies have identified additional genes that can be involved in azole resistance, such as *ERG3*, *TAC1*, *MRR1*, *UPC2* and *PDR3*, some of which provide resistance to different levels of drug than others [15,16]. It is becoming increasingly clear that the diversity of adaptive mutations is more varied than previously thought [15].

Larger and larger experiments are beginning to really highlight this problem. A recent study using pooled populations of genetically barcoded yeast enabled ~500,000 evolutionary replicates to be performed simultaneously [17•]. The researchers were able to detect ~25,000 unique adaptive lineages that had improved in their ability to survive glucose limitation; this is orders of magnitude more than any previous study. While many of the lineages carried the same or similar adaptive mutations, the lineages carrying smaller effect beneficial mutations would not have been detected in studies that utilized fewer replicates and/or had less power to distinguish low-frequency adaptive mutations from sequencing errors (Figure 1a). These lower frequency adaptive mutants are likely to be important, given follow-up studies showing that the most adaptive lineages often possess two mutations, including one from this low-frequency category [18]. Additional experimental designs using barcodes or other high replicate approaches continue to improve our ability to collect a fuller spectrum of mutations that are adaptive in a given conditions [19–22]. For example, recent work evolved *S. cerevisiae* to 80 different chemical compounds and identified 1405 mutations in 137 genes that provided resistance to at least one compound [20]. While several of these mutations were in known targets of adaptation, the most frequently hit genes were transcription factors, many of which had not previously been associated with drug resistance. While the focus of this review is on yeast as a model organism, we note that extensive experimental evolution data in bacteria also support the diversity of adaptive solutions [23–25]. These examples demonstrate there are many mutations available to solve an evolutionary challenge, making predictability in any given case that much harder.

Problem 2: the genetic basis of adaptation changes with genetic background

Adding further complications, new adaptive mutations can interact with existing genetic variation in unpredictable ways (i.e. epistasis, Figure 1b) [26•–28]. For example, several studies have shown that if an organism is already relatively fit, subsequent beneficial mutations will have a diminishing impact. This observation may reflect a global constraint, as fitness cannot increase linearly indefinitely — there are ceilings and floors on fitness (i.e. diminishing returns epistasis) [29–31]. However, recent studies suggest diminishing returns epistasis can also arise from idiosyncratic interactions among mutations to a small number of genes [32,33•]. Other studies of epistasis have shown more generally that the impact of a mutation can differ across genetically diverse strains [26•,34,35]. For example, Jerison et al. [34] evolved 230 yeast offspring that each differ by approximately 25,000 base pairs, finding that fitness effects of adaptive mutations were different in different offspring. A related complication is that the effects of subsequent mutations can depend on those that emerged in a previous round of adaptation, meaning that the outcome of adaptation becomes increasingly unpredictable with each successive mutation that fixes in the population [9,36–38]. Similarly, multiple mutations might arise in the same lineage in a short time period (relative to the generation time of the organism). Nonbeneficial mutations that appear concurrent with adaptive genotypes can rise in frequency in a population in a phenomenon called hitchhiking. The stochastic appearance of these hitchhiker mutations reduces the reproducibility of evolution and thus its predictability [39–41].

Problem 3: seemingly similar adaptive mutants have diverse and unpredictable effects on phenotype

In addition to the unpredictable interactions between adaptive mutations and their genetic backgrounds, the phenotypic effects of adaptive mutations can also be surprisingly difficult to predict [42•–44•]. This lack of predictability at the phenotypic level exists despite several studies showing that the mutations that help an organism survive a particular stress tend to fall into genes with similar functions [18,45], despite suggestion that the phenotypic basis of adaptation should be less complex than the genetic basis [42•,46], and despite strategies in evolutionary medicine that aim to exploit predictability at the phenotypic level [47,48]. But why are the phenotypic effects of adaptive mutants so unpredictable? More specifically, why do mutations that are all similarly adaptive in one "home" environment

behave differently in other "non-home" environments (Figure 1c)?

Several recent studies find that mutations are often pleiotropic in that they do not necessarily affect only a single trait [42•–44•,49–51]. Pleiotropy can negatively impact predictions about the fitness of mutations in novel environments, if the suite of traits affected by each mutant differs. For example, Kinsler et al. investigated the number of phenotypes individual adaptive mutations can affect by measuring the fitnesses of hundreds of yeast strains adapted to a single environment in a range of non-home environments. They found that very similar seeming adaptive mutations to negative regulators of the same pathway, or even within the same gene, can affect different sets of phenotypes and thus behave dissimilarly in non-home environments [42•]. This idea was reaffirmed by the experimental evolutions of Bakerlee et al. which demonstrated that lineages evolved in one condition had divergent fitness trajectories in new environments [43•]. Surprisingly, some lineages are more fit in non-home environments than their home environments, which may suggest evolving in the home environment is not necessarily the only or even the fastest way to adapt to that condition [42•,43•,51]. These studies highlight that the effects of adaptive mutations in non-home environments can be difficult to predict.

Why trying to predict adaptation is a worthwhile endeavor, despite the three aforementioned problems

The many factors that confound predictions of adaptation may cast doubt on the usefulness and practicality of the endeavor, but we believe the benefits of such predictions have the potential to be wide-reaching. A better understanding of how small effect mutations, interactions with genetic background, and pleiotropy all affect adaptation will shed light on the evolution of complex traits, especially since polygenic models of adaptation and complex trait architecture are becoming increasingly prevalent [52–54]. The applications of these predictions will extend beyond evolutionary biology to fields as diverse as medicine, agriculture, and conservation, for example, potentially allowing us to predict how pathogens will adapt to a drug or how organisms will be affected by climate change [44•,55–58].

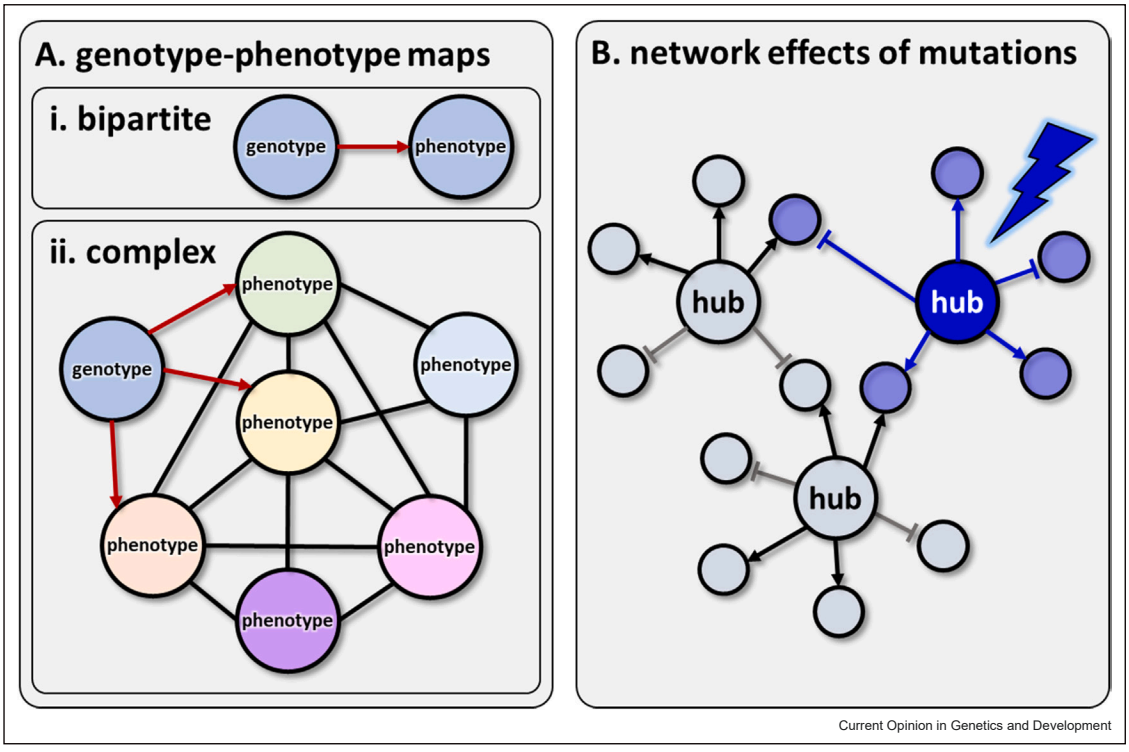
Strategies to make evolutionary predictions that take the complex genetic and phenotypic architecture of adaptation into account

We propose three potential, but not exclusive, solutions to the above problems when trying to predict adaptation. The first involves leveraging recent high-throughput technologies to collect richer data. This will inform us about how the effects of adaptation change with contexts like genetic background or environment. The second involves building more complex and accurate mathematical models of the genotype–phenotype map that take phenomena like epistasis and pleiotropy into account [59]. Finally, the third involves drawing insights from cell biology that can form the basis of physiological models that explain and predict the impacts of mutation on phenotypes and fitness [60–62].

Strategy 1: leveraging recent technologies to collect richer data

A key difficulty in predicting adaptation remains that we have not surveyed the possible adaptive mutations deeply enough, and that we do not understand the extent to which these adaptive solutions are equivalent (e.g. do all mutations behave similarly in all genetic backgrounds or environments). For example, while low replicate evolution experiments suggest mutations that resist one drug often induce ubiquitous sensitivity to a second drug, higher replicate studies uncover multiple resistance mechanisms with different tradeoffs [63•]. Thus, higher replicate evolution experiments are essential for comprehensively understanding the genetic architecture of adaptation. Formerly, performing very high replicate evolution experiments, and then surveying the behavior of diverse adaptive mutants in new environments and/or genetic backgrounds, presented a labor-intensive and time-consuming challenge. Happily, recent technologies, many of which were developed in budding yeast, allow this sort of rich experimental design. For example, the use of genomically integrated DNA barcodes has vastly expanded the number of replicate lineages that can be evolved in the laboratory [17•,37,43•,64–67], and has also hastened surveys of adaptive mutant fitnesses in diverse novel (e.g. non-home) environments [42•,43•,68]. Such surveys of how adaptive mutations impact fitness or phenotype can also be accomplished via high-throughput single-celled methods, such as microscopy [50] or emerging ultra-high-throughput single-cell RNA sequencing techniques [69•–73]. Yeast also leads the way in terms of surveying the impacts of adaptive mutations across diverse genetic backgrounds, mainly because genetically diverse strains can be easily mated to generate thousands of unique genetic recombinants [26•,34,74]. In sum, many technologies are emerging in yeast that allow high-throughput genome engineering [20,75] or recombinant strain construction [26•,34,74], high-replicate evolution [17•], and high throughput phenotyping and fitness measurement of adaptive mutants [42•,43•,68,69•]. These technologies are opening doors to long-standing questions about the repeatability of evolution, the predictability of adaptation, and the architecture of the genotype-phenotype map.

Figure 2



Illustrations of concepts described in Strategies 2 and 3. **(a)** genotype-to-phenotype maps. i. a simple bipartite model in which a single phenotype predictably correlates with genotype. ii. a more realistic, complex model in which genotype affects many correlated phenotypes through direct and indirect actions. **(b)** the network effects of mutations. Network models could help explain the pleiotropic and epistatic interactions of mutations. Detailing how genes interact can give us a basis for predicting mutational effects. For example, when a hub gene is hit with a mutation (blue), the effects of that mutation may extend to all of the genes with which it interacts.

Strategy 2: building more complex models of the genotype–phenotype map

In order to create predictive models of adaptation, it is necessary to develop novel mathematical frameworks that can analyze the humongous datasets generated by new methodologies described under Strategy 1. One approach involves examining the extent to which the phenotypic effects of different mutants are correlated in order to predict which mutants will behave similarly in novel contexts, rather than assuming that mutations that evolved in the same home environment will always have similar behavior in non-home environments (Figure 1c) [63•]. In the past, genotype-phenotype maps were usually represented by simple bipartite gene-trait maps (Figure 2a.i), which lacked the complexity to model the degree to which pleiotropic adaptive mutations affect overlapping or completely dissimilar groups of traits. Recently, several new models that allow pleiotropic genotype-phenotype maps and leverage the complex correlations among multiple phenotypes have been proposed (Figure 2a.ii) [42•,44•,50,76]. For example, models using a framework of correlated evolution of traits can be built to model the emergence of cross resistance or cross sensitivity to multiple drugs [44•,47,76]. In addition, correlative modeling techniques such as singular-value decomposition, principal component analysis, or machine learning can be applied to datasets that measure the fitness of different adaptive mutants across many environments in order to construct fitness predictions [42•,77,78]. A different framework for interpreting the massive amounts of data pertaining to adaptive mutants and their effects may involve meta-analysis comparing different approaches. For example, comparing different statistics used for studying the level of convergence among adaptive mutations helps better understand the mapping among mutations, phenotypes, and fitness during adaptation [46]. As more technologies emerge to yield more data about the genetic architecture of adaptation, more modeling approaches must follow.

Strategy 3: drawing insights from cell biology that can form the basis of physiological models

Building molecular and biochemical models informed by physiology also helps us understand the genetic architecture of adaptation and predict the effects of adaptive mutants in new genetic backgrounds and environments. For example, if a cell gains an adaptation to a high drug condition by overexpressing an efflux pump, we might

be able to predict that the cell will also have higher fitness in conditions with other environmental toxins. Since the mechanisms of adaptation often extend beyond a single pump, we need more complicated models incorporating more knowledge of the cell. Several types of modeling approaches have been considered. Here we will discuss two: network modeling and growth law theory.

Interaction networks summarize the connections among different elements in the cell (e.g. transcriptional networks, protein-protein interactions, etc.), and may provide insights about the effects of adaptive mutations. For example, consider a transcription factor that regulates stress-related genes. If it gains a mutation that affects its DNA binding dynamics to favor stronger expression, we might expect similar fitness gains under diverse stressful environments. Previous studies have suggested that adaptive mutations within the same network may improve fitness via similar phenotypic changes [18]. This intuition was supported by work showing that the beneficial effects of mutations in similar functional units were often not additive when combined, presumably because their effects are redundant [45,79]. But more recent work has revealed that even similar seeming adaptive mutations can have dissimilar fitness effects in non-home environments [42•,43•]. Thus, more complex network models are emerging that interrogate network structure to predict the epistatic and pleiotropic effects of mutation [59,80–82]. For example, the number of interacting partners (i.e. hubness) for a component, and the nature of these interactions (i.e. whether they are activating or repressing) (Figure 2b), may inform the amount of pleiotropy [79,83] and the amount and type of epistasis [84].

The network modeling mentioned above tends to require an exhaustive survey of genetic or metabolomic components per cell type. Opposingly, there are also attempts to use phenological models with less molecular-level details to model the physiology of microbes. One potential approach considers the 'growth laws', which model the rates of microbial growth as being set by one simple parameter: the percentage of proteome allocation to ribosomes [62,85–88]. For example, Scott et al. used a growth law model to predict how *E. coli* changes their growth rates when there is a ribosome-inhibiting antibiotic in the medium or ribosome-disrupting mutations in the genome [62]. Similarly, You et al. predicted the growth rates of *E. coli* under the disruption of signaling pathways critical for the starvation condition [88]. And Kavčič et al. predicted the growth-rate change of *E. coli* by the interactions between ribosome-inhibiting antibiotics and expression changes of translation-regulating genes [89•]. These examples highlight the potential usage of growth-law models to understand and predict interactions among adaptive mutations (posing a solution to Problem 2) and the environment-specific effects of adaptive mutations (posing a solution to Problem 3).

Conclusion

In conclusion, evolution is hard to predict. There are many routes to adaptation, and mutations can interact with existing genetic context and/or have unforeseeable effects on many phenotypes. However, we believe the expansion of high-throughput techniques, large data sets, and new mathematical and representative models that integrate these data can begin to explore the parameter space of adaptation, and generate predictive tools that will enable us to better forecast evolutionary trajectories in the future.

Conflict of interest statement

None.

Acknowledgements

We would like to thank the members of the NSF BII Mechanisms of Cellular Evolution Journal Club for helping us think about this topic. This work was supported by National Institutes of Health grant R35GM133674 (to KGS), an Alfred P Sloan Research Fellowship in Computational and Molecular Evolutionary Biology grant FG-2021-15705 (to KGS), and by a National Science Foundation Biological Integration Institution grant 2119963 (to KGS).

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest

1. van Leeuwen Jolanda, Pons Carles, Tan Guihong, Zi Yang Wang Jason, Hou Jing, Weile Jochen, Gebbia M, Liang W, Shuteriqi Ermira, Li Zhijian, Lopes Maykel, Ušaj Matej, Dos Santos Lopes Andreia, van Lieshout N, Myers C, Roth F, Aloy P, Andrews B, Boone Charles: **Systematic analysis of bypass suppression of essential genes**. *Mol Syst Biol* 2020, **16**:e9828.

2. Payen C, Di Rienzi SC, Ong GT, Pogachar JL, Sanchez JC, Sunshine AB, Raghuraman MK, Brewer BJ, Dunham MJ: **The dynamics of diverse segmental amplifications in populations of *Saccharomyces cerevisiae* adapting to strong selection**. *G3 Genes Genomes Genetics* 2013, **4**:399-409.

3. Quinto-Alemany D, Canerina-Amaro A, Hernández-Abad LG, Machín F, Romesberg FE, Gil-Lamaignere C: **Yeasts acquire resistance secondary to antifungal drug treatment by adaptive mutagenesis**. *PLoS One* 2012, **7**:e42279.

4. Bloom JS, Ehrenreich IM, Loo WT, Lite T-LV, Kruglyak L: **Finding the sources of missing heritability in a yeast cross**. *Nature* 2013, **494**:234-237.

5. Albert FW, Bloom JS, Siegel J, Day L, Kruglyak L: **Genetics of trans-regulatory variation in gene expression**. *eLife* 2018, **7**:e35471.

6. Nguyen BaAN, Lawrence KR, Rego-Costa A, Gopalakrishnan S, Temko D, Michor F, Desai MM: **Barcoded bulk QTL mapping reveals highly polygenic and epistatic architecture of complex traits in yeast**. *eLife* 2022, **11**:e73983.

7. Toprak E, Veres A, Michel J-B, Chait R, Hartl DL, Kishony R: **Evolutionary paths to antibiotic resistance under dynamically sustained drug selection**. *Nat Genet* 2012, **44**:101-105.

8. Brown KM, Costanzo MS, Xu W, Roy S, Lozovsky ER, Hartl DL: **Compensatory mutations restore fitness during the evolution of dihydrofolate reductase**. *Mol Biol Evol* 2010, **27**:2682-2690.

9. Costanzo MS, Brown KM, Hartl DL: **Fitness trade-offs in the evolution of dihydrofolate reductase and drug resistance in *Plasmodium falciparum***. *PLoS One* 2011, **6**:e19636.

10. Bazie VB, Ouattara AK, Sagna T, Compaore TR, Soubeiga ST, Sorgho PA, Yonli AT, Simpore J: **Resistance of *Plasmodium falciparum* to sulfadoxine-pyrimethamine (Dhfr and Dhps) and artemisinin and its derivatives (K13): a major challenge for malaria elimination in West Africa**. *J Biosci Med* 2020, **8**:82-95.

11. Chaturvedi R, Chhibber-Goel J, Verma I, Gopinathan S, Parvez S, Sharma A: **Geographical spread and structural basis of sulfadoxine-pyrimethamine drug-resistant malaria parasites**. *Int J Parasitol* 2021, **51**:505-525.

12. Roux AT, Maharaj L, Oyegoke O, Akoniyon OP, Adeleke MA, Maharaj R, Okpeku M: **Chloroquine and sulfadoxine–pyrimethamine resistance in Sub-Saharan Africa — A review**. *Front Genet* 2021, **12**:668574.

13. Cowen LE, Sanglard D, Calabrese D, Sirjusingh C, Anderson JB, Kohn LM: **Evolution of drug resistance in experimental populations of *Candida albicans***. *J Bacteriol* 2000, **182**:1515-1522.

14. Flowers SA, Colón B, Whaley SG, Schuler MA, Rogers PD: **Contribution of clinically derived mutations in ERG11 to azole resistance in *Candida albicans***. *Antimicrob Agents Chemother* 2015, **59**:450-460.

15. Nishimoto AT, Sharma C, Rogers PD: **Molecular and genetic basis of azole antifungal resistance in the opportunistic pathogenic fungus *Candida albicans***. *J Antimicrob Chemother* 2020, **75**:257-270.

16. Prasad R, Nair R, Banerjee A: **Multidrug transporters of *Candida* species in clinical azole resistance**. *Fungal Genet Biol* 2019, **132**:103252.

17. • Levy SF, Blundell JR, Venkataram S, Petrov DA, Fisher DS, Sherlock G: **Quantitative evolutionary dynamics using high-resolution lineage tracking**. *Nature* 2015, **519**:181-186.
Describes a new system for increasing the number of adaptive lineages and mutations that can be tracked during a laboratory evolution experiment.

18. Venkataram S, Dunn B, Li Y, Agarwala A, Chang J, Ebel ER, Geiler-Samerotte K, Hérissant L, Blundell JR, Levy SF, *et al.*: **Development of a comprehensive genotype-to-fitness map of adaptation-driving mutations in yeast**. *Cell* 2016, **166**:1585-1596.e22.

19. Ho W-C, Behringer MG, Miller SF, Gonzales J, Nguyen A, Allahwerdy M, Boyer GF, Lynch M: **Evolutionary dynamics of asexual hypermutators adapting to a novel environment**. *Genome Biol Evol* 2021, **13**:evab257.

20. Yeh C-LC, Tsouris A, Schacherer J, Dunham MJ: **High-throughput functional analysis of natural variants in yeast**. *bioRxiv* 2021,433108, https://doi.org/10.1101/2021.02.26.433108

21. Brauer MJ, Huttenhower C, Airoldi EM, Rosenstein R, Matese JC, Gresham D, Boer VM, Troyanskaya OG, Botstein D: **Coordination of growth rate, cell cycle, stress response, and metabolic activity in yeast**. *Mol Biol Cell* 2008, **19**:352-367.

22. Avecilla G, Chuong JN, Li F, Sherlock G, Gresham D, Ram Y: **Neural networks enable efficient and accurate simulation-based inference of evolutionary parameters from adaptation dynamics**. *PLoS Biol* (5) 2022, **20**:e3001633, https://doi.org/10.1371/journal.pbio.3001633 3001633, PMC9140244.

23. Good BH, McDonald MJ, Barrick JE, Lenski RE, Desai MM: **The dynamics of molecular evolution over 60 000 generations**. *Nature* 2017, **551**:45-50.

24. Lenski RE, Wiser MJ, Ribeck N, Blount ZD, Nahum JR, Morris JJ, Zaman L, Turner CB, Wade BD, Maddamsetti R, *et al.*: **Sustained fitness gains and variability in fitness trajectories in the long-term evolution experiment with *Escherichia coli***. *Proc R Soc B Biol Sci* 2015, **282**:20152292.

25. Wiser MJ, Ribeck N, Lenski RE: **Long-term dynamics of adaptation in asexual populations**. *Science* 2013, **342**:1364-1367.

26. • Vázquez-García I, Salinas F, Li J, Fischer A, Barré B, Hallin J, Bergström A, Alonso-Perez E, Warringer J, Mustonen V, *et al.*: **Clonal heterogeneity influences the fate of new adaptive mutations**. *Cell Rep* 2017, **21**:732-744.
Demonstrates how adaptive mutations have impacts that depend on genetic background.

27. Card KJ, Thomas MD, Graves JL, Barrick JE, Lenski RE: **Genomic evolution of antibiotic resistance is contingent on genetic background following a long-term experiment with *Escherichia coli***. *Proc Natl Acad Sci USA* 2021, **118**:e2016886118.

28. Marad DA, Buskirk SW, Lang GI: **Altered access to beneficial mutations slows adaptation and biases fixed mutations in diploids**. *Nat Ecol Evol* 2018, **2**:882-889.

29. Kryazhimskiy S, Rice DP, Jerison ER, Desai MM: **Global epistasis makes adaptation predictable despite sequence-level stochasticity**. *Science* 2014, **344**:1519-1522.

30. Johnson MS, Martsul A, Kryazhimskiy S, Desai MM: **Higher-fitness yeast genotypes are less robust to deleterious mutations**. *Science* 2019, **366**:490-493.

31. Lukačišinová M, Fernando B, Bollenbach T: **Highly parallel lab evolution reveals that epistasis can curb the evolution of antibiotic resistance**. *Nat Commun* 2020, **11**:3105.

32. Reddy G, Desai MM: **Global epistasis emerges from a generic model of a complex trait**. *eLife* 2021, **10**:e64740.

33. • Bakerlee CW, Nguyen Ba AN, Shulgina Y, Rojas Echenique JI, Desai MM: **Idiosyncratic epistasis leads to global fitness–correlated trends**. *Science* 2022, **376**:630-635.
Suggests an alternate mechanistic basis for diminishing returns epistasis.

34. Jerison ER, Kryazhimskiy S, Mitchell JK, Bloom JS, Kruglyak L, Desai MM: **Genetic variation in adaptability and pleiotropy in budding yeast**. *eLife* 2017, **6**:e27167.

35. Dutta A, Dutreux F, Schacherer J: **Loss of heterozygosity results in rapid but variable genome homogenization across yeast genetic backgrounds**. *eLife* 2021, **10**:e70339.

36. Starr TN, Flynn JM, Mishra P, Bolon DNA, Thornton JW: **Pervasive contingency and entrenchment in a billion years of Hsp90 evolution**. *Proc Natl Acad Sci USA* 2018, **115**:4453-4458.

37. Aggeli D, Li Y, Sherlock G: **Changes in the distribution of fitness effects and adaptive mutational spectra following a single first step towards adaptation**. *Nat Commun* 2021, **12**:5193.

38. Fisher KJ, Kryazhimskiy S, Lang GI: **Detecting genetic interactions using parallel evolution in experimental populations**. *Philos Trans R Soc B Biol Sci* 2019, **374**:20180237.

39. Lang GI, Rice DP, Hickman MJ, Sodergren E, Weinstock GM, Botstein D, Desai MM: **Pervasive genetic hitchhiking and clonal interference in forty evolving yeast populations**. *Nature* 2013, **500**:571-574.

40. Nguyen Ba AN, Cvijović I, Rojas Echenique JI, Lawrence KR, Rego-Costa A, Liu X, Levy SF, Desai MM: **High-resolution lineage tracking reveals travelling wave of adaptation in laboratory yeast**. *Nature* 2019, **575**:494-499.

41. Good BH, Desai MM: **The impact of macroscopic epistasis on long-term evolutionary dynamics**. *Genetics* 2015, **199**:177-190.

42. • Kinsler G, Geiler-Samerotte K, Petrov DA: **Fitness variation across subtle environmental perturbations reveals local modularity and global pleiotropy of adaptation**. *eLife* 2020, **9**:e61271.
Reveals how very similar adaptive mutants behave differently in non-home environments and creates a mathematical model that helps predict these disparate effects on fitness.

43. • Bakerlee CW, Phillips AM, Nguyen Ba AN, Desai MM: **Dynamics and variability in the pleiotropic effects of adaptation in laboratory budding yeast populations**. *eLife* 2021, **10**:e70918.
Reveals how adaptive mutants from the same home environment behave differently in non-home environments.

44. • Ardell SM, Kryazhimskiy S: **The population genetics of collateral resistance and sensitivity**. *eLife* 2021, **10**:e73250.
Uses a mathematical model to describe when mutants have predictable behavior in non-home environments vs. when their behavior is varied.

45. Tenaillon O, Rodríguez-Verdugo A, Gaut RL, McDonald P, Bennett AF, Long AD, Gaut BS: **The molecular diversity of adaptive convergence**. *Science* 2012, **335**:457-461.

46. Yeaman S, Gerstein AC, Hodgins KA, Whitlock MC: **Quantifying how constraints limit the diversity of viable routes to adaptation**. *PLoS Genet* 2018, **14**:e1007717.

47. Nichol D, Jeavons P, Fletcher AG, Bonomo RA, Maini PK, Paul JL, Gatenby RA, Anderson ARA, Scott JG: **Steering evolution with sequential therapy to prevent the emergence of bacterial antibiotic resistance**. *PLoS Comput Biol* 2015, **11**:e1004493.

48. Chen G, Mulla WA, Kucharavy A, Tsai H-J, Rubinstein B, Conkright J, McCroskey S, Bradford WD, Weems L, Haug JS, *et al.*: **Targeting the adaptability of heterogeneous aneuploids**. *Cell* 2015, **160**:771-784.

49. Bleuven C, Landry CR: **Molecular and cellular bases of adaptation to a changing environment in microorganisms**. *Proc R Soc B Biol Sci* 2016, **283**:20161458.

50. Geiler-Samerotte KA, Li S, Lazaris C, Taylor A, Ziv N, Ramjeawan C, Paaby AB, Siegal ML: **Extent and context dependence of pleiotropy revealed by high-throughput single-cell phenotyping**. *PLoS Biol* 2020, **18**:e3000836.

51. Jerison ER, Nguyen Ba AN, Desai MM, Kryazhimskiy S: **Chance and necessity in the pleiotropic consequences of adaptation for budding yeast**. *Nat Ecol Evol* 2020, **4**:601-611.

52. Boyle EA, Li YI, Pritchard JK: **An expanded view of complex traits: from polygenic to omnigenic**. *Cell* 2017, **169**:1177-1186.

53. Höllinger I, Pennings PS, Hermisson J: **Polygenic adaptation: from sweeps to subtle frequency shifts**. *PLoS Genet* 2019, **15**:e1008035.

54. Fagny M, Austerlitz F: **Polygenic adaptation: integrating population genetics and gene regulatory networks**. *Trends Genet* 2021, **37**:631-638.

55. Nosil P, Flaxman SM, Feder JL, Gompert Z: **Increasing our ability to predict contemporary evolution**. *Nat Commun* 2020, **11**:5592.

56. Lässig M, Mustonen V, Walczak AM: **Predicting evolution**. *Nat Ecol Evol* 2017, **1**:1-9.

57. Razgour O, Forester B, Taggart JB, Bekaert M, Juste J, Ibáñez C, Puechmaille SJ, Novella-Fernandez R, Alberdi A, Manel S: **Considering adaptive genetic variation in climate change vulnerability assessment reduces species range loss projections**. *Proc Natl Acad Sci USA* 2019, **116**:10418-10423.

58. Radchuk V, Reed T, Teplitsky C, van de Pol M, Charmantier A, Hassall C, Adamík P, Adriaensen F, Ahola MP, Arcese P, *et al.*: **Adaptive responses of animals to climate change are most likely insufficient**. *Nat Commun* 2019, **10**:3109.

59. Eguchi Y, Bilolikar G, Geiler-Samerotte K: **Why and how to study genetic changes with context-dependent effects**. *Curr Opin Genet Dev* 2019, **58–59**:95-102.

60. Domingo J, Baeza-Centurion P, Lehner B: **The causes and consequences of genetic interactions (epistasis)**. *Annu Rev Genom Hum Genet* 2019, **20**:433-460.

61. Drummond DA, Wilke CO: **Mistranslation-induced protein misfolding as a dominant constraint on coding-sequence evolution**. *Cell* 2008, **134**:341-352.

62. Scott M, Gunderson CW, Mateescu EM, Zhang Z, Hwa T: **Interdependence of cell growth and gene expression: origins and consequences**. *Science* 2010, **330**:1099-1102.

63. • Nichol D, Rutter J, Bryant C, Hujer AM, Lek S, Adams MD, Jeavons P, Anderson ARA, Bonomo RA, Scott JG: **Antibiotic collateral sensitivity is contingent on the repeatability of evolution**. *Nat Commun* 2019, **10**:334.
Demonstrates how higher replicate evolution experiments reveal a more varied spectrum of adaptive mutants that have dissimilar phenotypes.

64. Boyer S, Hérissant L, Sherlock G: **Adaptation is influenced by the complexity of environmental change during evolution in a dynamic environment**. *PLoS Genet* 2021, **17**:e1009314.

65. Venkataram S, Kuo H-Y, Hom EFY, Kryazhimskiy S: **Mutualism-enhancing mutations dominate early adaptation in a microbial community**. *bioRxiv* 2022,451547, https://doi.org/10.1101/2021.07.07.451547

66. Jasinska W, Manhart M, Lerner J, Gauthier L, Serohijos AWR, Bershtein S: **Chromosomal barcoding of *E. coli* populations reveals lineage diversity dynamics at high resolution**. *Nat Ecol Evol* 2020, **4**:437-452.

67. Li Y, Petrov DA, Sherlock G: **Single nucleotide mapping of trait space reveals pareto fronts that constrain adaptation**. *Nat Ecol Evol* 2019, **3**:1539.

68. Li Y, Venkataram S, Agarwala A, Dunn B, Petrov DA, Sherlock G, Fisher DS: **Hidden complexity of yeast adaptation under simple evolutionary conditions**. *Curr Biol* 2018, **28**:515-525.e6.

69. • Kuchina A, Brettner LM, Paleologu L, Roco CM, Rosenberg AB, Carignano A, Kibler R, Hirano M, DePaolo RW, Seelig G: **Microbial single-cell RNA sequencing by split-pool barcoding**. *Science* 2021, **371**:eaba5257.
Develops a novel single-cell RNA sequencing platform with the potential to reveal the phenotypic effects of many mutations across many conditions simultaneously.

70. Jackson CA, Castro DM, Saldi G-A, Bonneau R, Gresham D: **Gene regulatory network reconstruction using single-cell RNA sequencing of barcoded genotypes in diverse environments**. *eLife* 2020, **9**:e51254.

71. Jariani A, Vermeersch L, Cerulus B, Perez-Samper G, Voordeckers K, Van Brussel T, Thienpont B, Lambrechts D, Verstrepen KJ: **A new protocol for single-cell RNA-seq reveals stochastic gene expression during lag phase in budding yeast**. *eLife* 2020, **9**:e55320.

72. Nadal-Ribelles M, Islam S, Wei W, Latorre P, Nguyen M, de Nadal E, Posas F, Steinmetz LM: **Sensitive high-throughput single-cell RNA-Seq reveals within-clonal transcript-correlations in yeast populations**. *Nat Microbiol* 2019, **4**:683-692.

73. Urbonaite G, Lee JTH, Liu P, Parada GE, Hemberg M, Acar M: **A yeast-optimized single-cell transcriptomics platform elucidates how mycophenolic acid and guanine alter global mRNA levels**. *Commun Biol* 2021, **4**:1-10.

74. Liu X, Liu Z, Dziulko AK, Li F, Miller D, Morabito RD, Francois D, Levy SF: **iSeq 2.0: a modular and interchangeable toolkit for interaction screening in yeast**. *Cell Syst* 2019, **8**:338-344.e8.

75. Sharon E, Chen S-AA, Khosla NM, Smith JD, Pritchard JK, Fraser HB: **Functional genetic variants revealed by massively parallel precise genome editing**. *Cell* 2018, **175**:544-557.e16.

76. Gjini E, Wood KB: **Price equation captures the role of drug interactions and collateral effects in the evolution of multidrug resistance**. *eLife* 2021, **10**:e64851.

77. Wang X, Zorraquino V, Kim M, Tsoukalas A, Tagkopoulos I: **Predicting the evolution of *Escherichia coli* by a data-driven approach**. *Nat Commun* 2018, **9**:3562.

78. Tareen A, Kooshkbaghi M, Posfai A, Ireland WT, McCandlish DM, Kinney JB: **MAVE-NN: learning genotype-phenotype maps from multiplex assays of variant effect**. *Genome Biol* 2022, **23**:98, https://doi.org/10.1101/2020.07.14.201475

79. Costanzo M, Baryshnikova A, Bellay J, Kim Y, Spear ED, Sevier CS, Ding H, Koh JLY, Toufighi K, Mostafavi S, *et al.*: **The genetic landscape of a cell**. *Science* 2010, **327**:425-431.

80. New AM, Lehner B: **Harmonious genetic combinations rewire regulatory networks and flip gene essentiality**. *Nat Commun* 2019, **10**:1-12.

81. Kryazhimskiy S: **Emergence and propagation of epistasis in metabolic networks**. *eLife* 2021, **10**:e60200.

82. Kuzmin E, VanderSluis B, Wang W, Tan G, Deshpande R, Chen Y, Usaj M, Balint A, Usaj MM, Leeuwen J van, *et al.*: **Systematic**

analysis of complex genetic interactions. *Science* 2018, **360**:eaao1729.

83. Stern DL, Orgogozo V: **Is genetic evolution predictable?** *Science* 2009, **323**:746-751.

84. Geiler-Samerotte K, Sartori FMO, Siegal ML: **Decanalizing thinking on genetic canalization**. *Semin Cell Dev Biol* 2019, **88**:54-66.

85. Monod J: **The growth of bacterial cultures**. *Annu Rev Microbiol* 1949, **3**:371-394.

86. Scott M, Hwa T: **Bacterial growth laws and their applications**. *Curr Opin Biotechnol* 2011, **22**:559-565.

87. Metzl-Raz E, Kafri M, Yaakov G, Soifer I, Gurvich Y, Barkai N: **Principles of cellular resource allocation revealed by condition-dependent proteome profiling**. *eLife* 2017, **6**:e28034.

88. You C, Okano H, Hui S, Zhang Z, Kim M, Gunderson CW, Wang Y-P, Lenz P, Yan D, Hwa T: **Coordination of bacterial proteome with metabolism by cyclic AMP signalling**. *Nature* 2013, **500**:301-306.

89. • Kavčič B, Tkačik G, Bollenbach T: **Mechanisms of drug interactions between translation-inhibiting antibiotics**. *Nat Commun* 2020, **11**:4013.
Uses a growth-law model to predict microbial growth rates across diverse antibiotics.