

Epistasis and Adaptation on Fitness Landscapes

Claudia Bank^{1,2}

¹Institute of Ecology and Evolution, University of Bern, Bern, Switzerland;
email: claudia.bank@unibe.ch

²Swiss Institute of Bioinformatics, Lausanne, Switzerland

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Keywords

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Abstract

Epistasis occurs when the effect of a mutation depends on its carrier's genetic background. Despite increasing evidence that epistasis for fitness is common, its role during evolution is contentious. Fitness landscapes, which are mappings of genotype or phenotype to fitness, capture the full extent and complexity of epistasis. Fitness landscape theory has shown how epistasis affects the course and the outcome of evolution. Moreover, by measuring the competitive fitness of sets of tens to thousands of connected genotypes, empirical fitness landscapes have shown that epistasis is frequent and depends on the fitness measure, the choice of mutations for the landscape, and the environment in which it was measured. In this article, I review fitness landscape theory and experiments and their implications for the role of epistasis in adaptation. I discuss theoretical expectations in the light of empirical fitness landscapes and highlight open challenges and future directions toward integrating theory and data and incorporating ecological factors.

1. INTRODUCTION

Determining the relationship between genotype and fitness is a fundamental question in evolutionary biology and beyond. The concept of the fitness landscape quantifies this relationship. It provides an intuitive picture of how selection can direct evolution, iconically visualized by Wright (1932) as a topographic map with peaks and valleys. In a fitness landscape, populations evolve on fitness-increasing paths (via beneficial substitutions) until they reach a fitness peak (the fittest genotype in its mutational neighborhood). A rugged fitness landscape has multiple adaptive peaks representing different, sometimes suboptimal, stable genetic solutions to the challenge of surviving (and thriving) in a given environment. A necessary condition for the existence of more than one adaptive peak is epistasis for fitness, which occurs when one or more mutations interact (Wright 1931). (Throughout this review, I often refer to “epistasis for fitness” as “epistasis.”) Thus, questions about the shape of the fitness landscape are tightly linked to questions about the prevalence of epistasis.

The role of epistasis in evolution is controversial in the subfields of evolutionary research. Whereas epistasis is considered an important component of reproductive isolation between species when it occurs as hybrid incompatibility (Seehausen et al. 2014), it is usually not considered in studies of population and quantitative genetics. For example, population-genetic selection screens assume that the effect of a selected mutation is independent of the genetic background it occurs on, whereas in quantitative genetics, epistasis tends to appear as a noise term (Walsh & Lynch 2018). That is because epistasis is considered a transient, second-order effect that matters only at intermediate allele frequencies (Whitlock et al. 1995). Moreover, incorporating epistasis into evolutionary studies is difficult because of the complexity of multilocus interactions, resulting in theoretical and statistical challenges (Mackay 2013). Conversely, in molecular and systems biology, epistasis is considered a natural consequence of the structural interaction of mutations within a protein and the interaction of proteins in biological pathways (Domingo et al. 2019). The research area of fitness landscapes bridges evolutionary and systems biology and places epistasis at the heart of its study (Fragata et al. 2019). Fitness landscape theory provides expectations of how epistasis affects evolutionary trajectories, and empirical fitness landscapes provide information on the prevalence of epistasis in nature that feeds back into theory development.

In this review, I take a fitness landscape view of the role of epistasis in evolution. Section 2 highlights how epistasis constrains adaptation in two classical but different classes of fitness landscape models, and introduces how genotype–phenotype or phenotype–fitness maps can contribute to epistasis and its change across environments. Sections 3 and 4 review various empirical fitness landscape studies and evidence for epistasis in evolutionary trajectories that have occurred in nature. I then highlight empirical evidence for environment-dependent epistasis and how it is being integrated into fitness landscape models in Section 5. Section 6 concludes with open questions and a call for new approaches that incorporate ecological factors into the study of fitness landscapes.

2. FITNESS LANDSCAPE THEORY HIGHLIGHTS THE ROLE OF EPISTASIS IN EVOLUTION

The ideal genotype–fitness landscape maps every possible genotype to its carrier’s fitness. Even for small genomes, the resulting sequence space is much larger than the number of molecules in the known universe (Szendro et al. 2013). The topological features (e.g., the possibility of fitness ridges between two genotypes) of such a high-dimensional space are difficult to intuit or illustrate visually (e.g., McCandlish 2011). Although no experiment will be able to map the complete fitness landscape of an organism, mathematical models can capture this space and quantify the expected evolutionary patterns for a given shape of a fitness landscape. Fitness landscape theory quantifies

both the invariant properties of the complete fitness landscape (e.g., How many peaks and valleys are there? How frequent is epistasis?) and the evolutionary dynamics on the fitness landscape (e.g., Which peaks can be reached with what probability? How many steps does it take on average to reach a fitness peak?) and characterizes how they vary with the model parameters (e.g., the strength of epistasis).

In the following subsections, I introduce two classes of fitness landscape models: probabilistic genotype–fitness landscape models with few parameters, in which epistasis is tunable, represented by the rough Mount Fuji (RMF) model (Aita et al. 2000), and Fisher’s geometric model (FGM) (Fisher 1930), which creates an epistatic genotype–fitness landscape through its phenotype–fitness mapping. The two approaches highlight different modeling perspectives: Whereas probabilistic fitness landscapes directly map genotypes to fitness with a given amount of epistasis, FGM (as defined by Martin et al. 2007) ignores the possibility that epistasis could occur in the mapping of genotype to phenotype. Here, epistasis arises as a consequence of stabilizing selection for a phenotypic optimum. This difference between the RMF model and FGM highlights a feature inherent to all modeling: The model is greatly influenced by the philosophy of its developer, which affects the results that can be obtained. For example, Fisher (1930) considered adaptation to be a process driven by additive substitutions of small phenotypic effects toward a single optimum, whereas Wright (1932) believed epistasis to be pervasive at the phenotype level and the fitness landscape to be rugged. Both RMF- and FGM-based approaches show how epistasis constrains the course of evolution and predict patterns that were observed in recent empirical studies of fitness landscapes.

2.1. Probabilistic Fitness Landscape Models Feature Tunable Epistasis

Probabilistic fitness landscape models allow for the definition of arbitrarily epistatic fitness landscapes with few parameters and therefore lend themselves to mathematical analysis of the consequences of epistasis (Krug 2021). They are agnostic to any mechanistic or molecular reasons for epistasis and are only based on probability distributions. The RMF model (Aita et al. 2000, 2001) is highlighted here as representative of this class of models [see de Visser & Krug 2014, Ferretti et al. 2016, and Hwang et al. 2018 for descriptions of the various other members of this class, including the prominent NK model (Kauffman & Weinberger 1989)]. In the RMF model, each locus has an additive effect, either taken to be a constant or drawn from a probability distribution, and each genotype is assigned an epistatic deviation from its additive expectation, drawn from a normal distribution with mean zero. The RMF model interpolates between a fully additive fitness landscape, in which each substitution contributes a fixed amount to its carrier’s fitness independent of the rest of the genome (i.e., there is no epistasis), and the so-called house-of-cards fitness landscape (Kauffman & Levin 1987), which maximizes epistasis by assigning a random fitness to each genotype (**Figure 1**). The difference between the two limiting cases highlights an essential consequence of epistasis: It makes the fitness of a genotype less predictable. For example, for a fitness landscape with $L = 10$ loci and two alleles per locus, resulting in $2^L = 1,024$ genotypes, only 10 measurements are required to correctly predict the fitness of all genotypes in an additive fitness landscape, whereas 1,024 measurements are necessary to fully determine the house-of-cards landscape.

Probabilistic fitness landscapes such as the RMF model are an ideal testing ground to study the evolutionary consequences of epistasis when the landscape is tuned from additive to epistatic (Neidhart et al. 2014). In the smoothest, nonepistatic landscape, there is only a single, global fitness peak, and any population will eventually climb this peak (**Figure 1a,f**). At the same time, the evolutionary history of independently evolved populations will tend to be different, since any order of substitutions is possible. With maximum epistasis, the RMF landscape is equivalent to the house-of-cards landscape and displays a large number of fitness peaks, which is on average $2^L/(L + 1)$

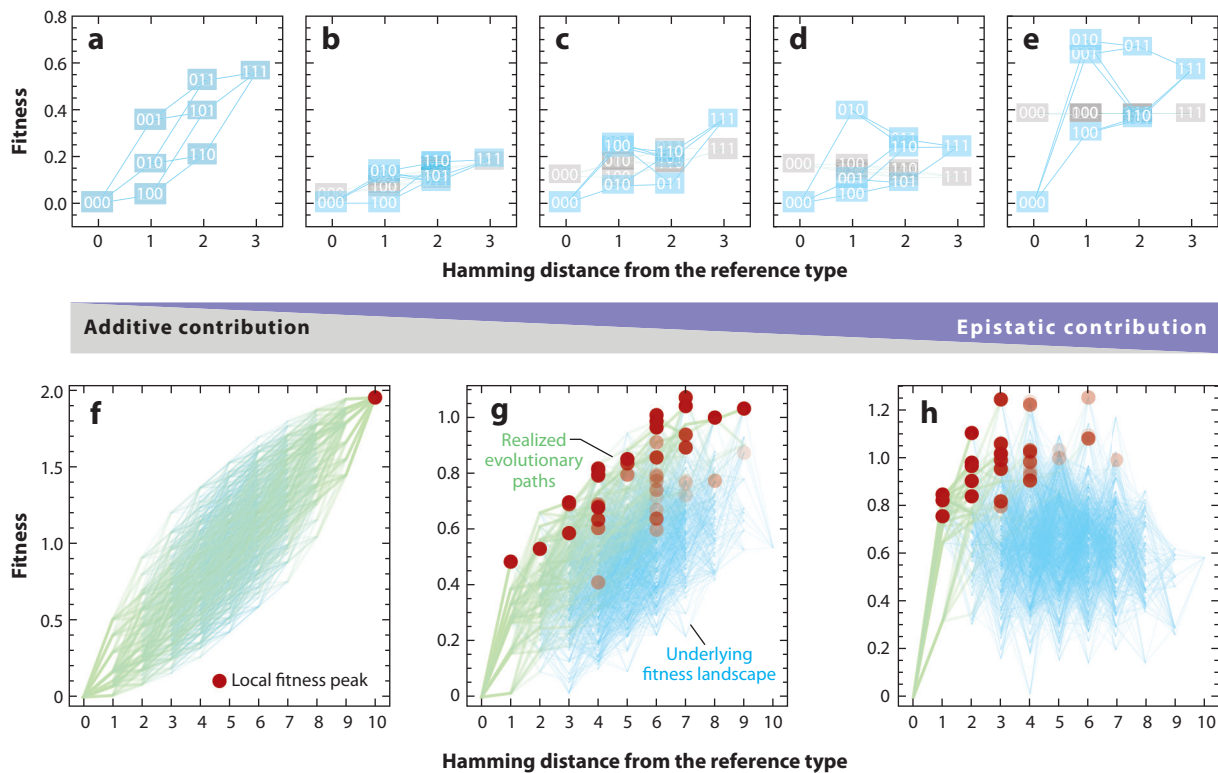


Figure 1

Illustration of the rough Mount Fuji (RMF) model. (a–e) Examples of three-locus RMF landscapes with increasing epistasis, ranging from (a) additive to (e) the house-of-cards model with maximum epistasis. The genotypes are ordered on the x axis by their Hamming distance (i.e., the number of substitutions distinguishing two genotypes) from the least fit reference genotype 000 (numbers in *white* indicate genotypes, where 0 refers to the reference allele at a locus and 1 to the mutated allele), whose relative fitness is arbitrarily normalized to zero. Single mutational steps are indicated by blue lines; blue boxes indicate binary genotypes. The underlying additive contribution to the fitness landscapes is highlighted by gray boxes and lines. More fitness landscapes with the same parameters are shown in **Supplemental Figure 1**. (f–h) Evolutionary trajectories are greatly affected by epistasis. For three landscapes with 10 loci (i.e., $2^{10} = 1,024$ genotypes) with varying strength of epistasis, 1,000 simulations of random adaptive walks were performed, starting at the least fit genotype 000. The underlying fitness landscape, showing the fitness of all 1,024 genotypes and their connections by single-step substitutions, is depicted in blue. The evolutionary paths that were taken are drawn on the fitness landscape in light green; paths that were taken repeatedly are indicated by greater color intensity. Local fitness peaks that were reached are highlighted in red circles, with color intensity related to the frequency of how often this adaptive peak was reached (see also **Supplemental Figure 2**; the simulation code is available in the **Supplemental Material**). (f) In an additive fitness landscape, the single adaptive peak is reached via many different evolutionary trajectories. (g) At intermediate epistasis, many of the existing adaptive peaks can be reached, but evolutionary trajectories become constrained by epistasis. (h) At maximum epistasis, few evolutionary paths remain accessible, and only the local fitness landscape is explored by the adaptive walks.

Supplemental Material >

(Kauffman & Levin 1987) (**Figure 1e,h**). Overall, epistasis introduces the potential for parallel fitness evolution toward different adapted genotypes (local fitness peaks). However, an increasing number of peaks in the landscape decreases the length of adaptive trajectories, and less of the full landscape is explored by evolution. Consequently, well-adapted genotypes are the most divergent for intermediate ruggedness of the landscape (**Supplemental Figure 2**). Moreover, epistasis tends to create historical contingencies of adaptation. It limits the possible orders of substitutions and thus the number of evolutionary trajectories toward a fitness peak, making evolution from the

same starting point more repeatable and seemingly more predictable (Figure 1; Supplemental Figure 2). Notably, the regularity of the RMF landscape (and other probabilistic fitness landscape models; Hwang et al. 2018), in which every genotype has the same statistical properties, is an unrealistic assumption for real interaction networks between mutations or proteins. Nevertheless, the RMF model was shown to recover features of various experimental fitness landscapes (Szendro et al. 2013, de Visser & Krug 2014, Bank et al. 2016).

2.2. Epistasis Arises from a Nonlinear Phenotype–Fitness Map in Fisher’s Geometric Model

Phenotype–fitness models such as FGM (Fisher 1930; reviewed in Tenaillon 2014) provide a perspective on epistasis complementary to that of probabilistic fitness landscape models. Importantly, under FGM, mutations that are additive on the phenotypic level can be epistatic on the fitness level, because phenotype and fitness are connected by a nonlinear fitness function (Martin et al. 2007, Gros et al. 2009) (Figure 2a). In FGM, fitness is determined by the distance of a phenotype from a single phenotypic optimum in a multidimensional trait space, usually under the assumption of a Gaussian fitness function (i.e., there is stabilizing selection for an optimum phenotype). New mutations appear as jumps in a random direction originating in the current phenotype. A mutation

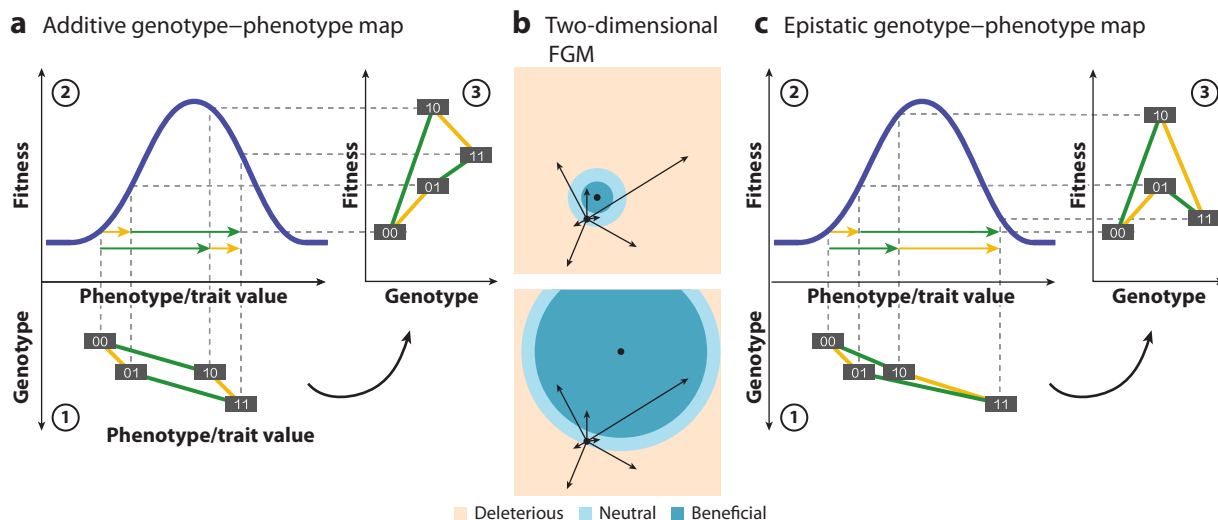


Figure 2

(a) In Fisher’s geometric model (FGM), a nonlinear phenotype–fitness map introduces epistasis on the genotype–fitness level, although mutations are additive on the genotype–phenotype level. The upper left quadrant ② shows a Gaussian phenotype–fitness relationship (blue curve) in a one-dimensional phenotype space. Two mutations are represented by yellow and green arrows. There is no epistasis on the genotype–phenotype level; in other words, the genotype–phenotype map is additive (lower left quadrant ①; the pairs of yellow and green lines, indicating single-step substitutions, are parallel and of equal length). The Gaussian phenotype–fitness function here results in negative epistasis on the genotype–fitness level (upper right quadrant ③; the pairs of yellow and green lines are at different angles and of different length). Binary genotypes are indicated by gray boxes. (b) In FGM, here visualized in a two-dimensional phenotype space, the same mutations (black arrows) have different fitness effects, determined as the distance gain or loss toward the optimum, when they occur close to (top) or far away from (bottom) the optimum (black dot). This scenario arises when the same mutations are tested on differently well-adapted genetic backgrounds in the same environment (resulting in epistasis) or when the same mutations are tested in environments with different phenotypic optima (resulting in genotype-by-environment interaction). (c) An epistatic genotype–phenotype map (lower left quadrant ①) and a nonlinear phenotype–fitness map with stabilizing selection for an optimum phenotype (blue curve in the upper left quadrant ②) interact to result in an epistatic genotype–fitness relationship (upper right quadrant ③). Binary genotypes are indicated by gray boxes, connected by single-step substitutions (yellow and green lines).

is considered beneficial when it decreases the distance from the phenotypic optimum. Epistasis occurs, for example, when the same mutation appears at different distances from the optimum (**Figure 2b**). FGM turns into a genotype–fitness landscape by introducing an explicit underlying genotype–phenotype map, allowing for the comparison of FGM-based and experimental fitness landscapes (Blanquart et al. 2014, Hwang et al. 2017). Interestingly, the single-peaked phenotype–fitness landscape of FGM can give rise to an underlying genotype–fitness landscape with multiple peaks (Hwang et al. 2017, Park et al. 2020, Srivastava & Payne 2022). The amount of epistasis for fitness at the genotype level depends on the distance from the fitness peak: Far away from the optimum, the same mutation tested on nearby genetic backgrounds tends to be nonepistatic, whereas strong epistasis can occur when the same mutation finds itself on nearby genetic backgrounds close to the phenotypic optimum. Interestingly, and importantly for experimental design, the sampling choice of mutations from the greater fitness landscape influences the shape of the combinatorially complete subset of genotypes built by those mutations: Individually beneficial substitutions, measured in a reference genetic background, will tend to result in a more epistatic fitness landscape in comparison to substitutions that occurred subsequently in an adaptive walk (Blanquart et al. 2014).

2.3. Modeling Assumptions and Challenges

The preceding subsections showed that in FGM epistasis arises from a nonlinear phenotype–fitness map, whereas RMF and other probabilistic fitness landscape models directly map genotype to fitness without any assumptions about the mapping of genotype to phenotype(s) to fitness. Importantly, epistasis for fitness can arise through both an epistatic genotype–phenotype map and a nonlinear phenotype–fitness map, and these levels may interact to yield a (potentially epistatic) genotype–fitness map (**Figure 2c**). This leads to an open question: How much epistasis for fitness can be attributed to an epistatic genotype–phenotype map, and how much is due to nonlinear phenotype–fitness relationships?

Ultimately, the genotype is connected to fitness by multiple layers of multiple phenotypes; thus, the picture discussed here and depicted in **Figure 2c** is greatly oversimplified. Kryazhimskiy (2021) recently conducted an elegant theoretical study of the propagation of epistasis across hierarchical levels in a metabolic network. They found that negative epistasis cannot be converted into positive epistasis at higher levels, whereas the opposite is possible. Interestingly, their study suggests that epistasis does not get diluted at higher levels but rather tends to become stronger.

Obtaining general mathematical results about evolution on theoretical fitness landscapes requires additional assumptions about the evolutionary dynamics. Most genotype–fitness landscape models address haploid, nonrecombining organisms in the limit of strong selection and weak mutation [i.e., neglecting neutral substitutions and assuming that mutations appear and fix sequentially (Gillespie 1984); for a review considering fitness landscape models with neutral substitutions and the consequences of genetic drift, see Fragata et al. 2019]. For example, when recombination is included in studies of adaptive walks on fitness landscapes, the connectedness of genotypes in the fitness landscape changes because recombination produces new combinations of (multiple) segregating alleles (Moradigaravand & Engelstädter 2012, Nowak et al. 2014). For rugged RMF landscapes, recombination creates an initial advantage to recombining populations that is later lost during adaptation because recombination dilutes genotypes necessary for crossing fitness valleys. Thus, theory predicts that recombining populations adapting on an epistatic fitness landscape can stall at local optima even more severely than nonrecombining populations (Nowak et al. 2014).

Studying diverse and recombining populations on fitness landscapes creates conceptual and technical questions. In a diploid fitness landscape, dominance has to be accounted for with respect to the direct effect of individual mutations and their epistasis because it affects both the duration

for which epistatic alleles segregate and their ultimate fate in a population (e.g., Turelli & Orr 2000, Blanckaert & Bank 2018). For example, currently there is no commonly used biologically reasonable and parameter-light definition of the dominance of epistasis, that is, of the effect of combinations of one or two copies of sets of epistatic alleles.

Finally, population dynamics considering multiple coexisting genotypes are difficult to characterize mathematically and to simulate computationally. If a starting population with standing genetic variation is to be considered, how should this population be distributed in the fitness landscape? Finding simple, computationally feasible, yet biologically relevant assumptions is a major challenge underlying theoretical studies of fitness landscapes.

3. EPISTASIS IS COMMON IN EXPERIMENTAL FITNESS LANDSCAPES

Recent advances in technology and experimental approaches [including CRISPR and site-directed mutagenesis to create sets of specific mutations (e.g., Hietpas et al. 2012, Karageorgi et al. 2019) and deep mutational scanning, potentially in combination with barcoding approaches, to measure phenotypes or fitness of thousands of genotypes in parallel (e.g., Fowler & Fields 2014, Johnson et al. 2019, Cote-Hammarlof et al. 2021)] have enabled the empirical assessment of increasingly large empirical fitness landscapes and the subsequent evaluation of fitness landscape theory. When selection is strong (e.g., in large microbial populations), and if these findings translate to natural environments, empirically tuned fitness landscape models promise to be useful for predicting evolution, which is relevant in medicine (e.g., antimicrobial resistance or cancer evolution) and with respect to climate change (e.g., potential for evolutionary rescue under the climate crisis) (de Visser & Krug 2014, Lässig et al. 2017, Wortel et al. 2021).

3.1. Engineered Fitness Landscapes Measured in the Laboratory

As an intermediate between theoretical fitness landscapes and data from nature, experimental fitness landscapes are appealing for several reasons. First, depending on the design of the mutant library, they may contain all genotypes of interest, not only those that are segregating naturally. Thus, deleterious or weakly beneficial genotypes can be measured, and inference of the selection coefficient (i.e., the relative competitive advantage of a genotype) can be performed for each genotype, resulting in a measurement of the (potentially combinatorially complete) fitness landscape. Second, the lab environment is controlled such that all genotypes grow or evolve in a constant, known, and similar environment. Finally, samples can be resequenced to check for secondary mutations, which in any event are minimized in the setup of deep mutational scanning, where populations are observed for only a few generations. Together, these features result in elegant and reproducible fitness data.

Many fitness landscape studies come from systems and molecular biology, where epistasis is considered a natural consequence of interactions within and between genes (reviewed in Phillips 2008, Athavale et al. 2014, Costanzo et al. 2019, Domingo et al. 2019). Because intra- and inter-genic interactions intuitively arise from the physical organization of proteins and the connection of genes in biological pathways, the question is not whether there is epistasis but rather how much, and which models of epistasis are most suitable. Among a multitude of studies that screened hundreds or thousands of engineered genotypes for (usually pairwise) epistasis, most focused on measurements of phenotypes that may be fitness related but do not directly translate into competitive fitness. Across these studies, pairwise epistasis was found to be common, and there is increasing evidence of higher-order epistasis (Domingo et al. 2019). Moreover, an increasing number of studies have followed evolutionary trajectories in the laboratory and dissected epistatic effects along those trajectories (e.g., Kryazhimskiy et al. 2014, Johnson et al. 2019). This review

focuses on engineered slices of fitness landscapes built from combinations of more than two mutational steps, with an emphasis on studies measuring competitive fitness.

3.2. Experimental Design Influences the Shape of the Fitness Landscape

Three main components distinguish experimental fitness landscape studies that may explain some of the observed differences between fitness landscapes. In addition, these different features of experimental fitness landscapes have direct implications for the connection of models and data.

3.2.1. What level of biological organization was considered? First, studies differ by the level of biological organization they consider. For example, a landmark study of all 32 combinations of five mutations in the beta-lactamase gene in *Escherichia coli* that together led to a 10^5 -fold increase in resistance to the antibiotic cefotaxime reported that only 18 out of 120 possible evolutionary trajectories from the reference genotype to the resistant genotype in the fitness landscape were accessible—that is, they had monotonically increasing fitness steps (Weinreich et al. 2006). In contrast, when Khan et al. (2011) performed competition experiments of the combinations of the first five adaptive substitutions that had been fixed during laboratory evolution of *E. coli* in a glucose-limited minimal medium, most of the possible evolutionary trajectories to the fittest genotype (86 of 120) were accessible. Here, Khan et al. (2011) studied substitutions in different genes, whereas Weinreich et al. (2006) studied a set of point mutations in the same gene.

On average, one might expect more epistasis between random alleles within the same gene than between random alleles across genes, because the physical structure of a protein leads to locally interacting residues and fine balances in the composition of the protein sequence. For example, Bank et al. (2015) reported extensive epistasis within the heat-shock protein Hsp90 in *Saccharomyces cerevisiae*, where almost 50% of $\sim 1,000$ interactions of pairs of point mutations in a nine-amino-acid region of the protein showed negative epistasis for competitive fitness, compared with $\sim 3\%$ of significant fitness interactions (based on colony size as measure of fitness) of double-gene knock-outs in the same species (Costanzo et al. 2010). These numbers have to be interpreted carefully: In addition to the fitness proxy, the measurement accuracy between these studies differed greatly. Moreover, an enrichment in epistasis is expected to occur more often between genes located in the same pathway than between random genes (Hurst et al. 2004). A systematic meta-analysis or a dedicated experimental study could better evaluate these expectations in the future.

Theoretically, knowledge of the structure of a protein, the interactions of genes within the protein, and the phenotypes important for fitness in a given environment could be used to develop predictive models of epistasis. Kemble et al. (2020) studied the competitive fitness of 1,369 genotypes in *E. coli* carrying mutations in two adjacent metabolic genes in the same pathway. The shape of their double-mutant fitness landscape strongly depended on the induced amount of gene expression in the pathway (which was tuned experimentally here and could represent different environmental pressures). This study stands out because the authors developed a specific model of the metabolic pathway that captured the severe changes in fitness effects and epistasis occurring across the different expression levels at impressive accuracy. Their model allowed for an accurate quantitative analysis of the specific scenario; at the same time, its specificity prevented straightforward extrapolation of the results to other pathways or higher-level questions such as the role of epistasis in evolution. Taking this approach to the organismal level, systems biologists have developed complex genome-scale metabolic models that predict phenotypes across environments (Bordbar et al. 2014, King et al. 2016). It would be interesting to see how such predictions relate to competitive fitness of bacterial strains in the lab or observed sequence variation in nature.

In general, the arguments above indicate that epistasis is expected to be most prevalent at short genomic distances, when substitutions within genes or in genes within the same pathway

interact. At the same time, the evolutionary relevance of epistasis at short physical distances in the genome is unclear. Even if variation were present at physically close loci, it would rarely be exposed to epistatic selection, as a result of the low recombination probability between closely linked loci and the low probability that the epistatic mutations occur in the same genotype. Evolutionary models of complex fitness landscapes incorporating recombination could shed light on how this dichotomy affects the expected distribution and role of epistatic variation in natural populations.

3.2.2. Which measure of fitness was used? Second, many studies have measured phenotypes other than competitive fitness, such as monoculture growth rate (e.g., Pinheiro et al. 2021), fluorescence (e.g., Sarkisyan et al. 2016), antibody binding (e.g., Adams et al. 2019), minimal inhibitory concentration in the presence of a drug (e.g., Weinreich et al. 2006), ribozyme activity (e.g., Bendixsen et al. 2021), or transcription factor binding (e.g., Aguilar-Rodríguez et al. 2017). Often, there is a clear relationship between the measured phenotype and reproductive success (e.g., if a protein is essential, its stability is necessary for survival of the individual). However, how the fitness-related phenotype maps to competitive fitness is generally unknown, and even bacterial growth rates in monoculture, which are often treated as equivalent to competitive fitness, are correlated with but do not translate directly into competitive fitness (Chevin 2011, Concepción-Acevedo et al. 2015). As shown in **Figure 2** and discussed in Section 2.3, epistasis on the genotype–phenotype level can differ greatly from epistasis for fitness, implying that genotype–phenotype landscapes may often be bad predictors of the true fitness landscape.

3.2.3. How were the genotypes for the fitness landscape chosen? Finally, the choice of mutation for an experimental fitness landscape affects its shape (Blanquart et al. 2014). Khan et al. (2011) combined five substitutions that had occurred and fixed together in an adaptive walk during laboratory evolution. Due to this choice, the five-step genotype was by definition highly fit, and at least one evolutionary trajectory between the reference and the endpoint had to be accessible. Using a different sampling strategy, Bank et al. (2016) studied the complete fitness landscape of 640 genotypes in *S. cerevisiae* of point mutations (all within a nine-amino-acid region in the heat-shock protein Hsp90) that had not previously been found together and individually ranged from beneficial to slightly deleterious. The resulting fitness landscape of up to six combined mutations showed both strong positive epistasis (at the local and global peaks) and strong negative epistasis, with many strongly deleterious combinations of individually beneficial mutations. Interestingly, none of the high-fitness genotypes observed in this fitness landscape were found in natural sequence data. The observed results were compatible with theoretical predictions: Mutations that occurred together in an adaptive walk were less epistatic than random mutations (Blanquart et al. 2014), there was an excess of positive epistasis at local peaks (Greene & Crona 2014), and negative (diminishing-returns) epistasis was common (discussed below in Section 3.3).

With increasingly large experimentally measured fitness landscapes, it has become possible to consider different levels of mutation choice in the same landscape. Pokusaeva et al. (2019) measured competitive fitness resulting in 12 fitness landscapes in *S. cerevisiae* that spanned more than four million genotypes, including the majority of extant amino acid substitutions of the *his3* protein across 21 yeast species. Their fitness landscapes were generally rugged, with 85% of substitutions depending on genetic background, and the evolutionary trajectory was constrained by epistasis. Whereas commonly observed and theoretically predicted patterns such as negative epistasis away from fitness peaks or ridges were confirmed in the large data set, simple models of sigmoidal phenotype–fitness relationships were not sufficient to describe the data; only a complex multilevel model captured the observed epistatic interactions.

3.3. Pervasive Diminishing-Returns Epistasis Points to Ubiquitous Interactions on the Genotype–Phenotype Level

Although the extent of inferred epistasis in experimental fitness landscapes differed between study system, measure of fitness, and environment, many studies shared the observation of negative (diminishing-returns) epistasis, where the effect of the same beneficial mutation was smaller when it was measured in a higher-fitness genetic background (for an earlier review, see de Visser & Krug 2014). Diminishing-returns epistasis results in a gradual slow-down of fitness evolution and is predicted across various fitness landscape models when the population approaches a fitness peak (Martin et al. 2007, Draghi & Plotkin 2013, Greene & Crona 2014, Lyons et al. 2020). Diminishing-returns epistasis is predicted generally during adaptation on a fitness landscape when there is widespread idiosyncratic epistasis, which corresponds to epistasis on the genotype–phenotype level in **Figure 2c** (Lyons et al. 2020, Reddy & Desai 2021). Models of idiosyncratic epistasis can, for example, explain the fitness trajectories obtained from a long-term evolution experiment in *E. coli*, in which, even after 50,000 generations of evolution, no fitness peak was reached (Wiser et al. 2013). Interestingly, the epistasis that occurs when an additive trait is translated into fitness through a nonlinear phenotype–fitness map [also termed nonspecific epistasis (Domingo et al. 2019); see, e.g., the genotype–phenotype–fitness map of FGM in **Figure 2a**] is not sufficient to guarantee persistent diminishing-returns epistasis (Reddy & Desai 2021). Thus, theory indicates that (potentially ubiquitous) epistasis on the genotype–phenotype level may contribute to observed evolutionary trajectories.

4. EPISTASIS AND FITNESS LANDSCAPES IN THE WILD

In an evolving population, epistasis is a second-order effect because it becomes visible only when recombination combines two (or more) epistatic alleles, or when mutation rates are high enough to create epistatic double mutants. Therefore, epistasis is generally believed to be negligible during adaptation on short timescales in comparison to other evolutionary forces, such as directional selection and genetic drift. However, the widely observed pattern of diminishing-returns epistasis, for example, can lead to a change in the selection gradient over time and is therefore likely to lead to incorrect predictions of how quantitative traits change over time (Hansen & Pélabon 2021). Moreover, epistasis creates detectable contingencies between substitutions in distantly related species that have been used to infer interactions between proteins (e.g., Morcos et al. 2011, Marmier et al. 2019). The prevalence of epistasis in experimental studies, together with its observed footprint in phylogenies, calls for a reevaluation of how epistasis affects evolutionary inference.

4.1. Experimental Evidence of Epistatic Adaptive Trajectories in the Wild

Retracing adaptive steps during evolution in the wild is becoming feasible with the advance of experimental methods, especially in organisms that lend themselves to experimental manipulation in the laboratory. However, few studies to date have explicitly screened evolutionary trajectories for the presence of epistasis for fitness (for a review of studies of genotype–phenotype epistasis, see Storz 2018). Pokusaeva et al. (2019), mentioned above, built intragenic fitness landscapes including extant variation across various yeast species. They found ubiquitous and often higher-order epistasis. The epistasis resulted in fitness ridges such that fit genotypes tended to be located near extant variation in the sequence space. Thus, epistasis most likely played a role during evolution by defining the accessible sequence space, including potentially neutrally accumulated divergence.

In a study of a protein under strong selection, Gong et al. (2013) and Gong & Bloom (2014) dissected the epistatic evolutionary trajectory of the human influenza nucleoprotein (NP) between

1968 and 2007. They used reverse genetics approaches to create genotypes carrying individual substitutions that had occurred during the evolutionary trajectory on the original genetic background from 1968, and used the growth rate of these genotypes in cell cultures as measure of their fitness. Gong et al. (2013) found that several of the substitutions that had fixed during the evolutionary history of NP were individually deleterious in the background of the original sequence but enabled the virus to subsequently accumulate beneficial immune system-evading substitutions. Interestingly, no epistatic trajectories were observed in NP in swine influenza (Gong & Bloom 2014). Whereas humans tend to get infected by influenza multiple times, swine are rarely infected more than once, leading to much stronger selection for immune invasion in human influenza than in swine influenza. The results from Gong et al. (2013) and Gong & Bloom (2014) indicate that strong selection was a prerequisite for observing epistasis in the evolutionary trajectory. Whereas laboratory studies in bacteria support that more strongly selected mutations have stronger interactions (e.g., Schenk et al. 2013), it remains unknown whether epistatic trajectories in nature are enriched for individually strongly selected substitutions. Whether this pattern is predicted by theory depends on the model scenario considered. For example, FGM predicts strong epistasis between strongly selected substitutions when the optimum is close, but not when it is far away.

An example of the dissection of an evolutionary trajectory in complex organisms comes from Karageorgi et al. (2019), who studied a convergent evolutionary trajectory of the adaptation of multiple insects, including monarch butterflies, to cardiac glycoside toxins. They overcame the challenges of retracing evolution by using CRISPR-Cas9-based genome editing, which allowed them to create and test combinations of the target mutations introduced into *Drosophila melanogaster*. They found strong positive epistasis for toxin tolerance among the three substitutions that had been repeatedly observed during evolution of toxin resistance. In addition, the order at which substitutions could accumulate was found to be constrained by epistasis (Karageorgi et al. 2019). Positive epistasis at fitness peaks is a general theoretical prediction that holds independently of the fitness landscape model (Greene & Crona 2014).

4.2. Epistasis Is Commonly Observed in the Form of Hybrid Incompatibility

In the research area of speciation, epistasis plays a major role when it manifests itself as hybrid incompatibility (reviewed in Seehausen et al. 2014, Coughlan & Matute 2020). The classical model that explains speciation via the evolution of postzygotic hybrid incompatibilities in geographically isolated populations is the (Bateson–)Dobzhansky–Muller model. In this model, allopatrically diverging populations accumulate substitutions over time. Any combinations of substitutions not tested by natural selection because they never segregated in the same population have the potential to be incompatible, leading to so-called Dobzhansky–Muller incompatibilities (DMIs). As pointed out by Gavrillets (1997), the resulting fitness landscape contains ridges of connected high-fitness genotypes and valleys of incompatible genotypes reflecting negative epistasis between the outermost genotypes on the ridge. Thus, a defining element of a classical DMI is that only one of the recombinant offspring of hybrids suffers from low fitness, whereas the broad definition of hybrid incompatibility includes any combination of alleles that reduces fitness in hybrids (Seehausen et al. 2014).

Hybrid incompatibilities and DMIs between species have been mapped both in laboratory crosses and, more recently, using large genomic screens, often revealing hundreds of pairwise incompatibilities between species pairs. For example, a genetic map of incompatibilities between the two fly species *D. melanogaster* and *Drosophila simulans* resulted in an estimate of more than 100 hybrid-lethal incompatibility regions (Presgraves 2003). Similarly, between two hybridizing swordtail fish in Mexico, genomic analyses of hybrid genomes indicated more than 100

incompatibility loci (Schumer et al. 2014). Also within species, segregating epistatic interactions may be common: Corbett-Detig et al. (2013) found various pairwise epistatic interactions when screening the global genetic variation within *D. melanogaster*.

Measuring a complex fitness landscape in the wild that encompasses more than pairwise interactions is difficult for various reasons. For example, diploidy creates a challenge for defining fitness, it is often difficult to obtain a sufficiently large sample to have a good representation of genotypes, fitness measurements are even less straightforward than in the lab, background variation might affect measurements, or experimental manipulation and creation of fitness variation can be impossible. Hybrid populations circumvent several of these challenges by being natural sources of genetic and fitness variation. Few studies to date have attempted to leverage this opportunity to map multilocus fitness landscapes in the wild. In a landmark study, Martin & Wainwright (2013) mapped the phenotype–fitness landscape of hybrids of three recently diverged pupfish ecotypes in field enclosures. On the basis of measures of growth and survival, they found a multi-peaked phenotype–fitness landscape isolating molluscivores from generalists with no evidence of frequency-dependent selection (Martin & Wainwright 2013, Martin 2016, Martin & Gould 2020). In a recent follow-up study, Patton et al. (2021) recently investigated the genotype–fitness landscape underlying the observed fitness differences between specialist and generalist phenotypes.

4.3. The Contentious Role of Epistasis During Speciation

Epistasis enables multiple genetic solutions to adaptation, represented by multiple peaks in the fitness landscape. Consequently, epistasis is thought to favor allopatric divergence. However, how much epistatic alleles contribute to speciation early in divergence or in the presence of gene flow, and how (much) epistatic variation is accumulated and maintained within populations, is an open question. Barton & Bengtsson (1986) argued that hybrid incompatibilities tend to be weak barriers to gene flow. Evolutionary theory has predicted that, because of the specific shape of their fitness landscape, epistatic alleles in a DMI cannot be maintained polymorphic in a population and that their accumulation in the presence of gene flow can only be a by-product, rather than a driver, of evolution (Gavrilets 1997, Bank et al. 2012). However, more complex epistatic interactions might behave differently. Blanckaert et al. (2020) recently showed that adding a third locus to the fitness landscape of a hybrid incompatibility allows for the evolution of complete reproductive isolation in the presence of gene flow (for a network-based approach, see Paixão et al. 2015). In a series of theoretical papers, Fraïsse et al. (2016), Simon et al. (2018), and Schneemann et al. (2020) studied the evolution of hybrid incompatibility using FGM, recovering many of the predictions of previous theoretical models and patterns observed in empirical data. Furthermore, they developed indices characterizing the respective influence of the environment and genetics on hybrid fitness (Schneemann et al. 2020).

Taking a fitness landscape view of speciation models in which higher-order interactions are possible is challenging due to the complexity of the models (and the potential genomic inference of such interactions), but it may be an opportunity to resolve the dichotomy between the frequency at which hybrid incompatibilities are observed and the theoretically ascribed minor role of epistatic loci early during divergence (Seehausen et al. 2014). Given the potential overrepresentation of epistatic loci at short physical distances in the genome (e.g., within proteins, or regulatory interactions), it will be important to address the role of genetic architecture and recombination distances in determining the evolutionary dynamics of epistatic loci (e.g., Blanckaert & Bank 2018). Here, probabilistic fitness landscape models can be a tool to quantify how the amount of epistasis in a fitness landscape affects speciation. For example, with increasing ruggedness, the number of fitness peaks increases and, with it, the potential for populations to climb different fitness peaks.

At the same time, these fitness peaks will be located close to one another in sequence space, which should result in a smaller barrier to gene flow when the diverged populations hybridize (see also **Supplemental Figure 2**). Thus, an intuitive prediction would be that intermediate epistasis maximizes the potential for stable divergence.

5. ENVIRONMENT-DEPENDENT EPISTASIS: MODELS AND EMPIRICAL EVIDENCE

5.1. Empirical Evidence for Environment-Dependent Epistasis

Despite great advances in the study of both theoretical and empirical fitness landscapes and epistasis, little is known about how fitness landscapes and epistasis change across environments. Whereas experimental screens have reported several cases of ubiquitous genotype-by-environment interactions for fitness ($G \times E$, also referred to as antagonistic pleiotropy and costs of adaptation; e.g., Bank et al. 2014, Flynn et al. 2020, Kinsler et al. 2020), the presence and magnitude of environment-dependent epistasis, or genotype-by-genotype-by-environment interactions ($G \times G \times E$) for fitness, have received little attention to date. The few existing examples have reported striking variation in epistasis across environments. When Hall et al. (2019) competed the 32 *E. coli* genotypes from Khan et al. (2011) across eight different environments, the resulting fitness landscapes looked vastly different between environments; epistasis was shown to be both strong and environment specific (see also Flynn et al. 2013) (**Figure 3**). In an experimental study

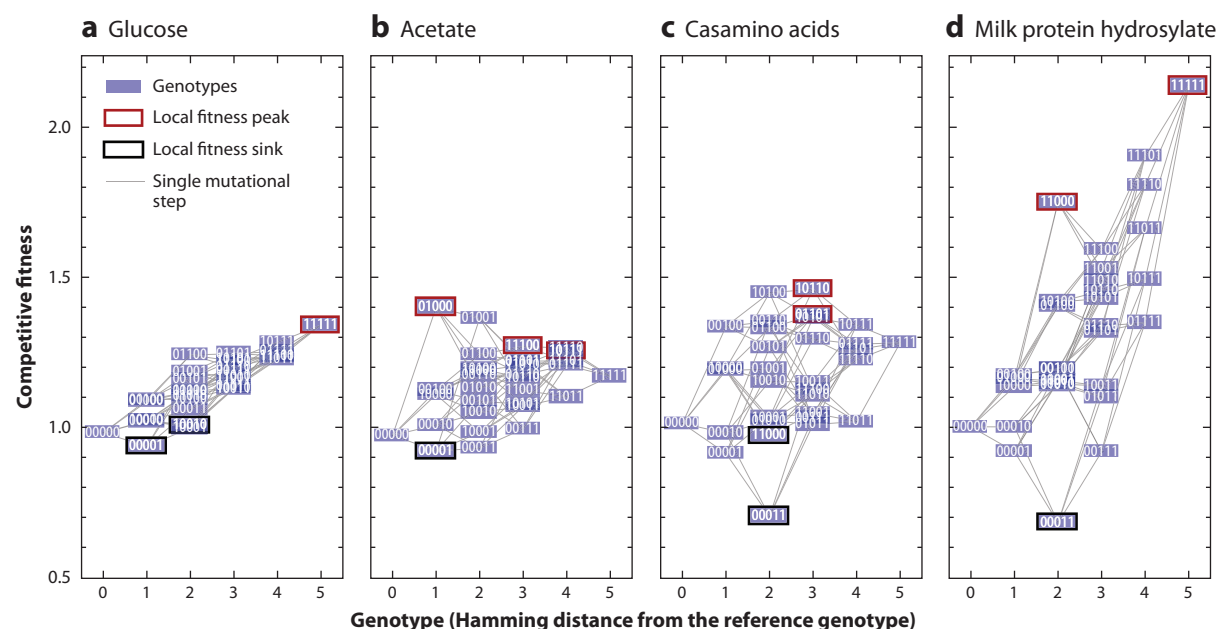


Figure 3

Environment-dependent fitness landscapes from Hall et al. (2019). Each fitness landscape consists of the same $2^5 = 32$ genotypes, initially studied by Khan et al. (2011). The five substitutions combined in this landscape had been the first steps of an adaptive walk in a glucose environment. Their competitive fitness was then measured in the original environment (a) and in seven additional environments (three of which are shown here in panels b–d). In each fitness landscape, single mutational steps are indicated by gray lines, blue boxes indicate genotypes, and local fitness peaks and sinks are highlighted in red and black, respectively. The fitness landscapes differ significantly across environments in terms of absolute effect sizes, location and number of peaks and valleys, and accessible evolutionary paths. The fitness landscape in the original evolution environment is the smoothest.

of yeast that had evolved in the presence of a fungicide, epistatic interactions between two point mutations could change greatly depending on the concentration of the drug (Ono et al. 2017). Gorter et al. (2018) showed that the evolution of yeast populations in the presence of increasing concentrations of nickel followed the evolutionary trajectory predicted by the changes in the fitness landscape along the dosage gradient. Lindsey et al. (2013) showed that environment-dependent epistasis prohibited evolutionary rescue of *E. coli* populations under high rates of environmental change. In a high-throughput study of a yeast tRNA fitness landscape across four environments, Li & Zhang (2018) also reported a widespread environment dependence of epistasis. Finally, Bakerlee et al. (2022) recently reported large environmental variation with respect to both single-mutation effects and pairwise epistasis of a fitness landscape spanning 10 mutations in different genes in yeast, measured in six environments. On the basis of their analysis, these authors argued that epistasis in their fitness landscapes arose from specific gene interactions rather than a global, nonspecific, nonlinear phenotype–fitness relationship.

Environment-dependent epistasis has also been observed in studies of insects and fish. Nosil et al. (2020) mapped the fitness landscape related to cryptic coloration observed in stick insects using a field experiment. Here, the fitness proxy was survival during a 2-day exposure to predators in the wild. In a subset of F2 offspring from a sympatric pair of three-spined stickleback populations that otherwise showed little evidence of epistatic interactions, Arnegard et al. (2014) identified an environment-dependent functional mismatch of oral jaw traits that contributed to a reduction in feeding performance. Interestingly, both studies pointed to a major role of environment-dependent changes in phenotype–fitness relationships in creating and altering epistasis.

5.2. Theoretical Approaches to Environment-Dependent Epistasis

Most fitness landscape theory to date has addressed a single, static environment and, thus, a static fitness landscape. However, the study of evolution in changing environments is important both from a basic science point of view and with respect to the response of populations to anthropogenic change and the climate crisis. Fluctuating phenotypic optima turn a fitness landscape into a “seascape” (Mustonen & Lässig 2009). Although the concept of the fitness seascape is gaining increasing interest from the scientific community, little research has addressed how to extend the existing fitness landscape theory to account for changing environments and how it affects epistasis and its consequences.

FGM is the most extensively studied class of fitness landscape models with respect to changing environments (Martin & Lenormand 2006, 2015; Zhang 2012; Matuszewski et al. 2014; Harmand et al. 2017; Schneemann et al. 2020). Different environments can be incorporated in FGM by assuming a change in the location of the phenotypic optimum (Martin & Lenormand 2006, 2015) (Figure 2*b*). Under this assumption, it is possible to study environmental gradients as well as completely different environments, and to develop expectations of the amount of $G \times E$ interaction. Using experimental data from *E. coli*, evolved at different concentrations of an antibiotic drug, Harmand et al. (2017) predicted the costs of antibiotic resistance across environmental gradients with generalized FGM. From both a theoretical and an experimental point of view, an exciting next step would be to determine how the underlying genotype–fitness landscape changes across environments.

Several studies of dynamic genotype–fitness landscape models have addressed how populations adapt to fluctuating environments, with a focus on the response to the timescale at which fluctuations occur and whether evolution results in the repeated switching between specialists or the evolution of generalists (Trubenová et al. 2019, Wang & Dai 2019, Sachdeva et al. 2020). Moreover, Agarwala & Fisher (2019) showed that in a slowly changing environment, epistasis promotes

evolutionary dynamics that switch between short phases of rapid adaptation and long periods of stasis in which the population waits for new adaptive paths to open up.

5.3. Environment-Dependent Epistasis Could Leave a Footprint of Ecological History in Genomes

Recent theoretical research by Das et al. (2020) has shown how simple genotype–phenotype relationships can result in environment-dependent epistasis. Their model specifically described the genotype–fitness landscapes along an environmental gradient of antibiotic concentration, based on Hill functions that are commonly used to describe the S-shaped relationship between bacterial growth rate and antibiotic concentration (so-called pharmacodynamics; Goutelle et al. 2008). Das et al. (2020) assumed that every genotype has a different S-shaped fitness curve and that there is a trade-off between growing well in the absence and the presence of the antibiotic; in other words, resistant genotypes must have a cost of resistance. Their model was in excellent agreement with a previously described experimental fitness landscape from *E. coli* (Marcusson et al. 2009). Interestingly, although Das et al. (2020) observed little epistasis for the two main phenotypic parameters of the model, the drug-free growth rate of genotypes and their IC_{50} (the concentration of the drug that reduces the growth rate by 50%), epistasis was common in the composite fitness measure combining these two parameters and varied along the dose gradient. Epistasis was greatest at intermediate concentrations of the antibiotic and the fitness ranking of the genotypes changed most rapidly at intermediate drug concentrations. The authors concluded that small changes in (intermediate) dosage might have large consequences for evolution, because new evolutionary routes may open up or close.

Extending their theoretical analysis of the model, Das et al. (2021) found that a genotype can carry information about its ecological history; in other words, the current genotype carries information about previous environments. This scenario arises in fitness landscapes in which epistatic relationships change along an environmental gradient, such that evolutionary trajectories are contingent on the previously experienced environment. First, some genotypes were accessible only at low doses of an antibiotic drug and would therefore never be observed if the population experienced high doses of the drug in its recent history. Second, there was on average limited reversibility of evolutionary paths; in other words, if the environmental change was reversed, then the population did not tend to take the reverse evolutionary path. Notably, the analytical results obtained are based on specific models and approaches inspired by theoretical physics. It will be interesting to see whether similar results can be obtained from other models incorporating environmental gradients, such as FGM.

6. CONNECTIONS, DISCREPANCIES, AND FUTURE CHALLENGES

6.1. Ecological Factors May Smoothen the Fitness Landscape That Matters for Evolutionary Dynamics in the Wild

Under the assumption of environmental fluctuations in natural environments, not only may evolution be constrained by epistasis but also this epistasis may be variable across environments, further complicating the study of selective forces that acted during evolutionary history. So far, few models can accommodate the change of epistasis across environments, and there is little knowledge about whether these effects could cancel out when integrated over evolutionary history, perhaps resulting in, on average, neutral-like evolutionary dynamics.

At the same time, selection coefficients inferred from empirical fitness landscape studies tend to be much larger than those estimated via inference of the distribution of fitness effects from polymorphism data (reviewed in Tataru & Bataillon 2020). This could be due to the complexity

of natural environments in comparison to experimental settings. Over evolutionarily relevant timescales (i.e., those reflecting the polymorphism used for the abovementioned inference methods), the environment experienced by a population has likely fluctuated greatly (including the effect of ecological interactions of the population with its environment), such that any specific strong fitness effect may be diluted to an average over the time and number of experienced environments (and fitness-related phenotypes). This view would reflect the seascape approach to fitness landscapes (Mustonen & Lässig 2009). A laboratory environment removes as many of these fluctuations and complexities as possible to extract the fitness (or phenotypic) effect of mutations in a well-defined environment. Thus, a main target of selection in the laboratory could be to streamline molecular processes and to remove all unnecessary mechanisms that allow an organism to respond to environmental fluctuations, for example, by knocking out genes with premature stop codons (e.g., Kvitek & Sherlock 2013, Kryazhimskiy et al. 2014). In addition, fluctuations could result both from a changing environment altering the fitness of genotypes and from population size changes altering the strength of genetic drift, thereby making fitness differences invisible. Just as Wright (1932) argued, population bottlenecks could effectively smoothen the shape of the fitness landscape perceived by an evolving population in nature.

In the extreme case, all but the strongest epistasis could become invisible to natural selection and the fitness landscape could effectively smoothen to an almost flat surface with holes of strongly deleterious, incompatible genotypes, as described in Gavrilets's (2004) holey fitness landscape model. Relatedly, the study of combinatorially complete fitness landscapes might be misleading our intuition about evolution on these landscapes. The epistasis experienced by an evolving population based on its segregating variation is likely different from the observed epistasis when considering the entire possible fitness landscape (Whitlock et al. 1995). Quantifying these differences and finding measures of "realized epistasis" are challenging, because the segregating variation strongly depends on evolutionary parameters such as ploidy, recombination, population size, and mutation rate.

6.2. At What Level Do Ecology and the Environment Modify Fitness Landscapes, and What Traces Does That Leave?

We do not know what part of the genotype–phenotype–fitness relationship is most affected by changes in the environment, or what causes changes in epistasis across environments (**Figure 4b–d**). Empirical and theoretical studies could address this question by developing models allowing for environment-tunable epistasis on the genotype–phenotype and phenotype–fitness levels and by designing approaches to extract these respective pieces of the fitness landscape. As **Figure 4b,c** shows, a change in the phenotype–fitness mapping straightforwardly leads to changes in the genotype–fitness landscape and, thus, changes in epistasis across environments. This can occur, for example, when a previously fitness-relevant phenotype becomes irrelevant in a different environment (e.g., camouflage in a dark or turbid environment, fast swimming in the absence of a predator, or antibiotic resistance in an antibiotic-free environment; **Figure 4c**). In contrast, the environment can change epistasis in the genotype–phenotype map through environmentally induced (potentially transgenerational) epigenetic modification without changing the phenotype–fitness map, for example, by silencing a gene involved in an epistatic interaction (**Figure 4d**). Epistasis constrains evolutionary trajectories. If it changes across environments, then this implies that genomes in the present may contain information about the environment(s) the population has experienced in the past. Thus, the inference of evolutionary history from genomic samples may be linked to (or confounded by) the ecological history of a population (Das et al. 2021). Currently we do not know whether and in what conditions this concept is applicable to more general fitness landscapes, or whether it can be translated into an inference framework for genomic data.

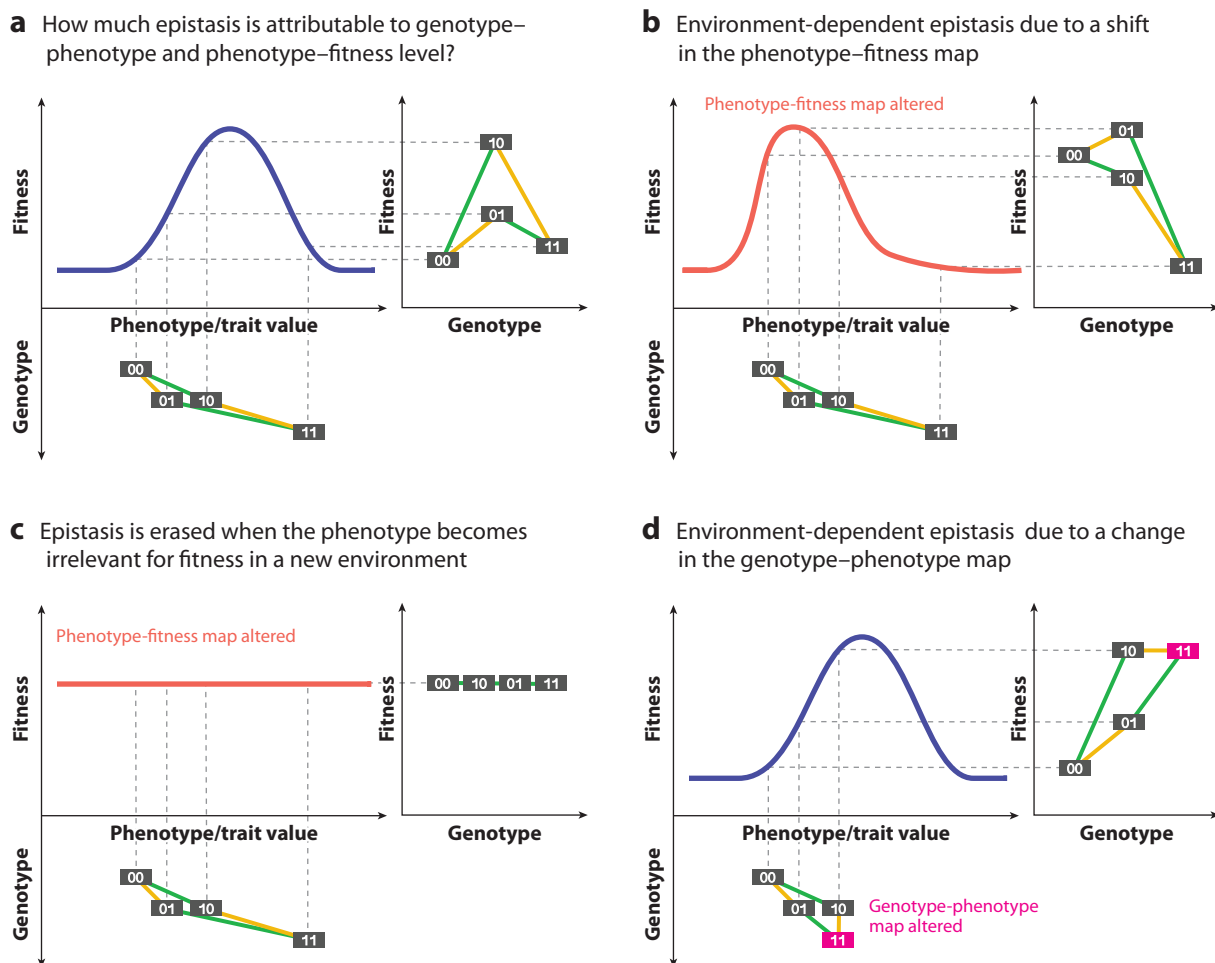


Figure 4

Challenges facing the dissection of genotype–fitness relationships and the study of environment-dependent epistasis. (a) Epistasis on the genotype–phenotype level interacts with nonlinear phenotype–fitness relationships. (b) A shift in the phenotypic optimum introduces environment-dependent epistasis. (c) Epistasis for fitness is erased when a trait becomes irrelevant for fitness in a new environment. (d) Epigenetic modification can introduce a change in the genotype–phenotype and genotype–fitness maps (pink box) without affecting the phenotype–fitness map. Each panel represents a simplified sketch of genotype–phenotype–fitness maps. The upper left quadrant shows a phenotype–fitness map (red or blue curve) in a one-dimensional phenotype space. The genotype–phenotype level is represented by two mutations (yellow and green lines) interacting epistatically (lower left quadrant; positive epistasis in panels a–c, negative epistasis in panel d). Binary genotypes are indicated by gray boxes, connected by single-step mutations (yellow and green lines).

Moreover, we do not know how the contingency of evolutionary trajectories caused by epistasis (in stable or fluctuating environments), and the frequency-dependent selection caused by epistatic selection in admixed populations, may confound population-genetic inference of evolutionary histories or selection. As a first step and a proof of concept to address this question, features of probabilistic fitness landscapes with widespread epistasis could be incorporated into genomic simulations to quantify how the presence of epistasis changes subsequent population-genetic inference.

Finally, epistasis can span all biological levels of organization: within or between genes, between host and symbiont, and possibly between species (e.g., Sørensen et al. 2021, Buskirk et al.

2020). Incorporating these levels into a theory of coevolutionary fitness landscape is an exciting challenge. One theoretical approach could be to concatenate the genomes of interacting species into one meta-genome as a node in the fitness landscape—but how would such a model reflect the potentially different generation times and population sizes of the interacting species? Also within species, competition for resources could lead to a dynamic fitness landscape in which the fitness of one genotype in a population depends on the frequency of the others. For example, epistasis on the genotype–trait level is likely to affect the long-term diversity of the population as it propagates into the (frequency-dependent) genotype–fitness map.

7. FINAL REMARKS

Epistasis and fitness landscapes are an active subject of study across different fields of biology. In evolutionary biology, this subject stands a bit isolated, if not esoteric, as the role of epistasis in evolution is often believed to be marginal except as a mechanism to maintain species integrity. It is time to acknowledge the prevalence of epistasis, which has left its marks in contingent evolutionary trajectories. New theoretical and empirical evidence of the extent and architecture of epistasis, and new possibilities to study complex interactions theoretically and empirically, call for a reevaluation of how epistasis affects (and has affected) evolution and whether and how epistasis might confound inferences from evolutionary studies. Moreover, evolutionary perspectives on genotype–phenotype–fitness relationships and the importance of environment-dependent epistasis are essential for further development of the biological relevance of fitness landscape studies. Bridging the gap between theoretical and experimental fitness landscapes and the role of epistasis in the wild requires interdisciplinary research integrating the viewpoints of molecular biology, systems biology, and ecology and evolution.

SUMMARY POINTS

1. Fitness landscape theory shows how epistasis constrains adaptation and promotes divergence, and indicates how experimental choices influence the expected epistasis in an empirical fitness landscape.
2. Epistasis is prevalent in empirical fitness landscapes, with a common pattern of diminishing-returns epistasis, where the beneficial effect of a substitution is smaller when it appears in a fitter genetic background.
3. There is increasing evidence that epistasis can be environment dependent. Theory indicates that genomes that evolved in a fitness landscape with environment-dependent epistasis may carry information in their ecological history.

FUTURE ISSUES

1. How can the existing fitness landscape theory be applied, generalized, or extended to cases of diploid/recombining/polymorphic/small populations that evolve in changing environments and in the presence of ecological interactions?
2. How much do the prevalence of epistasis and the resulting historical contingency affect evolutionary inference?

3. How frequently and in what way does the environment alter epistasis on the genotype–phenotype level and the phenotype–fitness level, respectively?

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Contents

Asymmetric Inheritance: The Diversity and Evolution of Non-Mendelian Reproductive Strategies <i>Laura Ross, Andrew J. Mongue, Christina N. Hodson, and Tanja Schwander</i>	1
Functional Roles of Parasitic Plants in a Warming World <i>David M. Watson, Richard C. McLellan, and Francisco E. Fontúrbel</i>	25
Evolution and Ecology of Parasite Avoidance <i>Amanda K. Gibson and Caroline R. Amoroso</i>	47
Fungal Dispersal Across Spatial Scales <i>V. Bala Chaudhary, Carlos A. Aguilar-Trigueros, India Mansour, and Matthias C. Rillig</i>	69
Local Adaptation: Causal Agents of Selection and Adaptive Trait Divergence <i>Susana M. Wadgymar, Megan L. DeMarche, Emily B. Josephs, Seema N. Sheth, and Jill T. Anderson</i>	87
Simulation Tests of Methods in Evolution, Ecology, and Systematics: Pitfalls, Progress, and Principles <i>Katie E. Lotterhos, Matthew C. Fitzpatrick, and Heath Blackmon</i>	113
Complexity, Evolvability, and the Process of Adaptation <i>David Houle and Daniela M. Rossoni</i>	137
Consistent Individual Behavioral Variation: What Do We Know and Where Are We Going? <i>Kate L. Laskowski, Chia-Chen Chang, Kirsten Sheehy, and Jonathan Aguiñaga</i>	161
Evolutionary Transitions Between Hermaphroditism and Dioecy in Animals and Plants <i>John R. Pannell and Crispin Y. Jordan</i>	183
Evolutionary Ecology of Fire <i>Jon E. Keeley and Juli G. Pausas</i>	203
Rethinking the Prevalence and Relevance of Chaos in Ecology <i>Stephan B. Munch, Tanya L. Rogers, Bethany J. Johnson, Uttam Bhat, and Cheng-Han Tsai</i>	227

Integrating Fossil Observations Into Phylogenetics Using the Fossilized Birth–Death Model <i>April M. Wright, David W. Bapst, Joëlle Barido-Sottani, and Rachel C.M. Warnock</i>	251
Cophylogenetic Methods to Untangle the Evolutionary History of Ecological Interactions <i>Wade Dismukes, Mariana P. Braga, David H. Hembry, Tracy A. Heath, and Michael J. Landis</i>	275
Evolution and Community Assembly Across Spatial Scales <i>Mathew A. Leibold, Lynn Govaert, Nicolas Loeuille, Luc De Meester, and Mark C. Urban</i>	299
The Unusual Value of Long-Term Studies of Individuals: The Example of the Isle of Rum Red Deer Project <i>Josephine M. Pemberton, Loeske E.B. Kruuk, and Tim Clutton-Brock</i>	327
The Macroevolutionary History of Bony Fishes: A Paleontological View <i>Matt Friedman</i>	353
The Evolution and Ecology of Interactions Between Ants and Honeydew-Producing Hemipteran Insects <i>Annika S. Nelson and Kailen A. Mooney</i>	379
One Health for All: Advancing Human and Ecosystem Health in Cities by Integrating an Environmental Justice Lens <i>Maureen H. Murray, Jacqueline Buckley, Kaylee A. Byers, Kimberly Fake, Elizabeth W. Lehrer, Seth B. Magle, Christopher Stone, Holly Tuten, and Christopher J. Schell</i>	403
Freshwater Fish Invasions: A Comprehensive Review <i>Camille Bernery, Céline Bellard, Franck Courchamp, Sébastien Brosse, Rodolphe E. Gozlan, Ivan Jarić, Fabrice Teletchea, and Boris Leroy</i>	427
Epistasis and Adaptation on Fitness Landscapes <i>Claudia Bank</i>	457

Errata

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