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REVIEWS

Review

Evolution in the light of fitness landscape theory

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By formalizing the relationship between genotype or phenotype and fitness, fitness landscapes harbor information on molecular and evolutionary constraints. The shape of the fitness landscape determines the potential for adaptation and speciation, as well as our ability to predict evolution. Consequently, fitness landscape theory has been invoked across the natural sciences and across multiple levels of biological organization. We review here the existing literature on fitness landscape theory by describing the main types of fitness landscape models, and highlight how these are increasingly integrated into an applicable statistical framework for the study of evolution. Specifically, we demonstrate how the interpretation of experimental studies with respect to fitness landscape models enables a direct link between evolution, molecular biology, and systems biology.

The fitness landscape – a useful concept for the study of evolution

A longstanding goal of evolutionary biology is to understand the relationship between genotype (see Glossary), phenotype, and fitness, and its consequences for adaptation and speciation. The theory of fitness landscapes encompasses a multitude of models that map genotype or phenotype to fitness [1–5]. Fitness landscape models greatly simplify the study of adaptation by reducing individuals to either genotypes or phenotypes, whose reproductive success is determined by a single trait, namely fitness. This simplification makes evolution tractable and potentially predictable [1,4–6]. However, reduction of the interaction of evolutionary forces and the complexity of nature to differences in fitness in a static environment can also lead to an incorrect assessment of possible evolutionary paths and constraints [4,7] (Box 1). Nevertheless, owing to its appealing simplicity, which lends itself to mathematical analysis, the concept of fitness landscapes has been widely used. Today we can draw from a large body of fitness landscape theory that was developed to investigate questions in speciation, adaptation, and molecular and systems biology (Fig. 1) (e.g., [2,3,5,8–10]).

For almost a century fitness landscapes have mostly been studied theoretically. Recent advances in next-generation sequencing now allow assessment of increasingly large experimental fitness landscapes with unprecedented accuracy (e.g., [11–15]; reviewed in [5]). This has spurred the development of methods to bridge the gap between theoretical and empirical fitness landscapes, and created the opportunity to evaluate the classical theory (e.g., [16–19]). Moreover, the flexibility of the fitness landscape concept allows the relationship between genotypes or phenotypes and fitness to be studied across biological levels (Fig. 2) [3,4,20]. This flexibility is reflected in the variety of questions to which fitness landscape theory has been applied, ranging from speciation to protein and gene network evolution (Fig. 1). Although the same overarching framework underlies this work, and similar models are often derived and analyzed, there is little exchange between these fields. Complementing other reviews, which

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have provided important in-depth overviews of the recent advances in either of these fields [5,20–22], we here highlight the connections and overlaps between the fields. Specifically, we emphasize several recent theoretical and empirical examples of interdisciplinary fitness landscape studies. These examples illustrate how fitness landscape models can be used to estimate the prevalence of epistasis, study the importance of **neutral** evolution, and address how integration of the genotype–phenotype map into the theory of fitness landscapes might allow for the prediction of evolution.

Epistasis in the light of fitness landscapes

The term epistasis, first introduced by Bateson [23,24], describes the fitness effects of mutations that are specific to the genetic background the mutation occurs on. From a fitness landscape point of view, epistasis represents a nonlinearity in the function that describes the fitness landscape. In the following we discuss how, through the study of epistasis, fitness landscape theory has been invoked in the study of speciation, adaptation, and biological networks.

Manifested as postzygotic genetic incompatibilities between species, epistasis maintains species barriers via selection against unfit hybrid offspring [25]. Indeed, one of the earliest descriptions of a fitness landscape model can be found in the work of Bateson [23]. It would later become known as the Bateson–Dobzhansky–Muller (BDM) model [26,27], arguably the most widely accepted model for the evolution of postzygotic reproductive isolation [28–30]. This model describes how genetic changes that accumulate in geographically isolated populations can be incompatible, in other words leading to unfit hybrid offspring when brought together in the same genome. The BDM model explains the buildup of reproductive isolation via genetic incompatibilities without the need to cross a fitness valley, in other words without the need to invoke the fixation or bypassing of deleterious intermediate steps during divergence [28]. Essentially, the BDM model specifies a fitness landscape in which there is a ridge of fit genotypes along which the populations evolve, but where the accumulated mutations from diverged populations interact negatively epistatically. Various BDM incompatibilities have been identified empirically (Fig. 1) [31–33]. Theoretical studies

Box 1. The problem of dimensionality and the illusion of crossing valleys.

Fitness landscapes are multidimensional. However, they are often visualized in 3D, two dimensions corresponding to genotypic or phenotypic axes, and a third to fitness (but see [6]). Such a representation leads to a wrong intuition of how populations evolve, and this has spurred criticism of the importance of the concept [7]. Specifically, the representation of the landscape in 3D leads to a picture with local peaks separated by valleys of low fitness, implying that switching fitness peaks requires crossing a fitness valley. This neglects the potential existence of high-fitness ridges that connect fitness peaks along other dimensions of the landscape. To an extreme, this is illustrated in the 'holey landscape model' [20] (Figure I). Considering only neutral and lethal genotypes, this model captures how populations can move along fitness ridges and end up at opposite ends of a fitness hole, leading to reproductive isolation without ever 'crossing' a valley.

The dimensionality of the fitness landscape is also important when considering experimental fitness landscapes. Indeed, it is impossible to capture the full fitness landscape of an organism or even of a single molecule. Therefore, we must focus on a subset of the full fitness landscape. It has been shown [18] that extrapolating any properties from the local fitness landscapes requires a rigorous statistical framework because many different global landscapes can give rise to the same local fitness landscape. To further illustrate this point we show that apparently unconnected networks of genes can be connected when considering the full fitness landscape (Table 1).

The number of dimensions of a 'holey fitness landscape' or a neutral network determines the percolation threshold P_t of said network [2,20], $p_t \approx 1/L$, a measure of the connectivity of the landscape. A fitness landscape is subcritical (i.e., composed of many small networks of fit genotypes, isolated from each other), if $P_v < P_t$, where P_v is the probability that a given genotype is viable. In this case, each genotype is trapped within its own vicinity. If $P_v < P_t$, a single massive network exists in which all fit genotypes are connected through single mutation steps.

Glossary

Biological network: The set of elements (e.g., genes) necessary for the realization of a biological or ecological function, and the various relationships (e.g., activation, inhibition) that exist between the elements of the set.
Epistasis: Genetic background-specific (fitness) effect of a mutation.
Effective population size: The hypothetical population size of a Wright–Fisher population (panmictic and of constant size) that best reproduces the observed population genetics statistics.
Evolvability: The ability of a biological system (a population, individual, network, or molecule) to have or produce variants that can be acted upon by selection.
Fitness: A measure of the reproductive or replicative success of a biological entity (from molecules to individuals). Usually, fitness-related phenotypes (e.g., growth rate) are used as proxies for fitness.
Genetic drift: Random change in allele frequencies over time in a population of finite size.
Genotype: The genetic constitution of an organism.
Genotype–fitness landscape: A map from (usually) discrete genotypes to fitness. Genotypes tend to represent nucleotide, amino acid, or gene differences.
Locus: A position in the genome of an individual. Depending on the focus of the study, a locus can correspond to a single nucleotide or amino acid position, several base pairs of DNA sequence, or a gene.
Mutation: A heritable change in the genetic sequence of an individual.
Neutral: A property of a mutation which harbors no fitness effect for an individual, such that its frequency in the population depends only on extraneous factors, such as genetic drift. A mutation is conditionally neutral if its neutrality is genetic-background or environment-dependent.
NK model: Mathematical model that describes a genotype–fitness landscape with different degrees of epistasis. This model is defined by the parameters N, the number of loci in the landscape, and K, the degree of epistasis between loci.

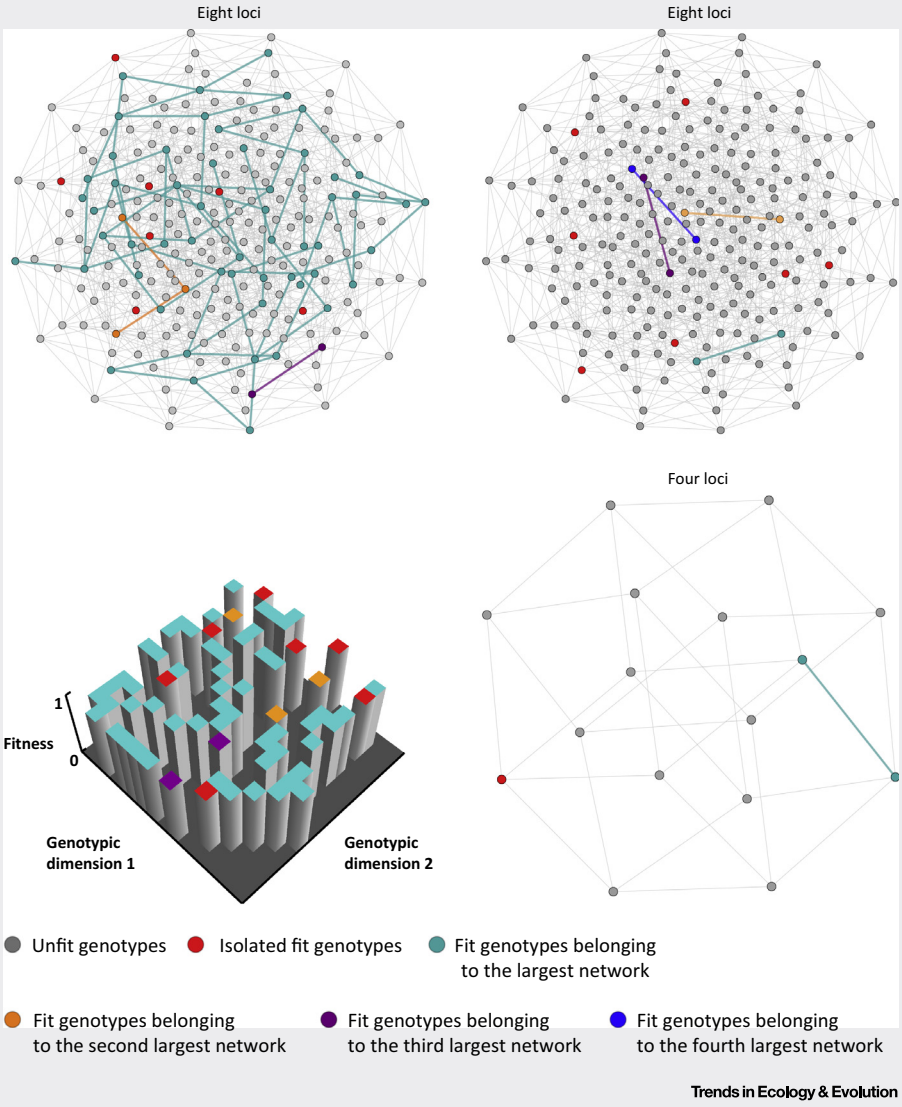


Figure I. Representation of a 'holey landscape' as a network (top) and 3D fitness landscape (bottom). We compare two cases in which the probability, $P_v = 0.2$, for a genotype to be fit, is below (left panels; $L = 4$ and therefore $P_t \approx 0.25$) and above (right panels: $L = 8$ and therefore $P_t \approx 0.125$) the percolation threshold. For the same proportion of fit versus unfit genotypes, larger landscapes are easier to explore: for $L = 4$, 66% of the fit genotypes are in the largest network, and for $L = 8$, 82% belong to the largest network. Colors indicate the different connected networks, with red indicating isolated genotypes. In the network view (upper panels), genotypes are connected by single step mutations. The figure illustrates how the 3D projection creates a wrong impression of fitness valleys by masking the connectivity that exists between fit genotypes in higher dimensions.

Phenotype: A set or subset of observable traits of an individual that stems from the interactions between genotype and environment.
Phenotype–fitness landscape: A map from (usually) continuous phenotypes to fitness. In a multidimensional phenotype space, each dimension is composed by a different one-dimensional trait.
Pleiotropy: The property of a gene to affect more than one independent phenotypic trait.
Rough Mount Fuji model (RMF): A model describing a genotype–fitness landscape that is composed of an additive component and an epistasic component. Ruggedness is then tuned by changing the relative proportions of the two components.

Table 1. Properties of subsampled fitness landscapes.[a]

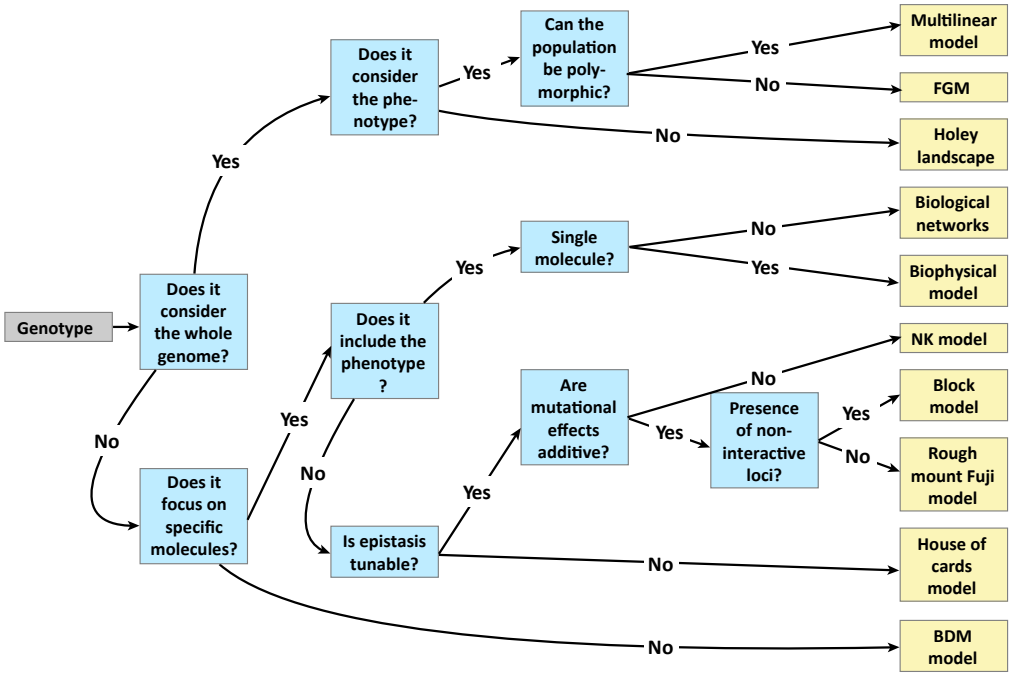
	$P_v = 0.5$	$P_v = 0.2$	$P_v = 0.1$	$P_v = 0.05$	$P_v = 0.01$
Probability of having at least two fit genotypes	0.999	0.874	0.494	0.197	0.008
Average number of networks	1.12	0.778	0.462	0.406	0.125
Probability of having at least two isolated networks	0.386	0.773	0.763	0.706	0.75
Probability that two isolated networks in the subsampled fitness landscape are connected in the full landscape	0.925	0.212	0.336	0.588	>5/6

[a]We simulated a 'holey fitness landscape' with eight diallelic loci and subsampled fitness landscape with four loci. P_v corresponds to the probability that a given genotype is fit, with the fitness of genotypes assigned through permutation. Here $P_t \approx 0.125$, therefore the first two columns correspond to supercritical networks, while the last three are subcritical networks. In the subsampled fitness landscapes, we estimated the number of isolated networks of genotypes (i.e., groups of fit genotypes that are not connected by fit genotypes). In addition, we quantified the probability that two isolated networks were connected in the full fitness landscape. Each measure was obtained based on 1000 simulations.

of the BDM model and its extensions (e.g., [34,35]) traditionally focus on small fitness landscapes. This is because one needs to define $2^L - 1$ parameters (one fitness for each genotype minus one reference) to fully specify the fitness landscape for L diallelic loci. A minimal genome-wide model of epistasis, the 'holey landscape model', was proposed by Gavrilets [20]: genotypes are either fit or lethal, with this fitness being assigned randomly, as illustrated in Box 1. The resulting fitness landscape is highly epistatic because the complete genotype information is necessary to know the fitness of a given genotype, and has been used to study speciation through genetic drift. In addition, this model highlights the importance of the dimensionality of fitness landscapes: higher-dimensional fitness landscapes tend to be more connected (i.e., lower probability of having truly isolated fitness peaks or ridges) than their lower-dimension counterparts (Box 1). In addition to its application to questions in speciation, the holey landscape model was also successfully applied to the study of RNA folding and structure [36], which illustrates the versatility of the model (also discussed in the next section).

During adaptation to a new environment, epistasis dictates which sequences of consecutive mutations are feasible [11]. For example, a recent study of the influenza nucleoprotein [37] confirmed the theory that adaptive functional changes to a protein frequently destabilize the protein structure, such that compensatory changes are necessary to restabilize the structure around the adaptive functional change [9,38]. Gong et al. [37] reconstructed an adaptive walk, and found that each activity-enhancing (i.e., functionally adaptive) mutation was followed by a mutation that increased the structural stability of the protein. When the authors introduced the activity-enhancing mutations without their stability-enhancing partners, growth of the virus was strongly impaired. In such cases the amount of epistasis in the fitness landscape limits the possible routes to high fitness (Box 2). In a growing body of theoretical work, several statistics have been developed to quantify the amount and type of epistasis in a given fitness landscape. How these relate to each other was analyzed using tunable theoretical fitness landscapes, most notably the NK model and the rough Mount Fuji model (RMF) [16,19].

Epistasis is also prevalent in phenotype–fitness landscape models, such as Fisher's geometric model (FGM) [8,39]. In FGM, populations evolve through mutations that are represented by jumps in an n-dimensional phenotype space. Mutations in FGM are usually assumed to be additive at the phenotype level. Nevertheless, mutational effects become epistatic at the fitness



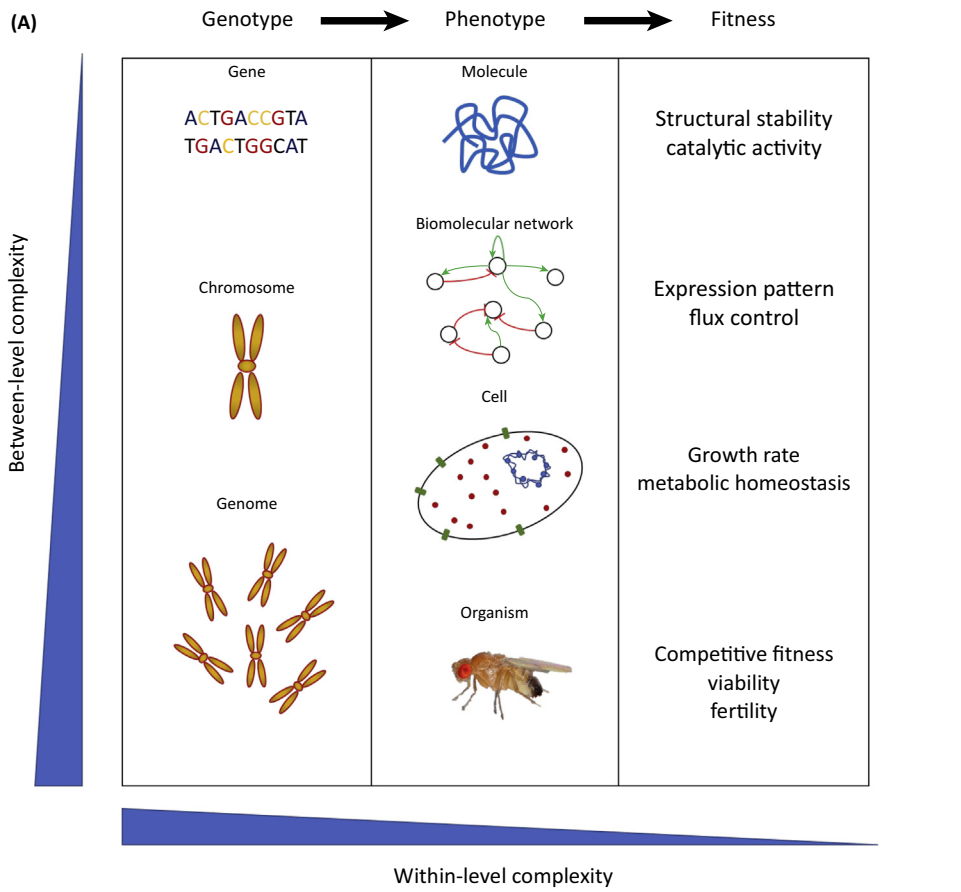
Theoretical examples	Experimental examples
[84, 85, S1, S2]	NA
[8, 10, 18, 39, 41, 42, 79-81, S3-S8]	[18, 40, 83, S9-S11]
[2, 36, 56, S12-S15]	[2, 36, S12, S13]
[48, 56, 74, 76-78, 111, S16,S17]	NA
[57, 62, S18-S21]	[47, 70]
[3, 17, 53, 54, 88, S22-S24]	[14,16]
[S25-S28]	[S29]
[54, 88, 95, S24, S30]	[14,16]
[95, S23, S24, S31-S33]	[14,16]
[23, 26-28, 34, S34-S43]	[31-33, 49, S44-S55]

Fig. 1. Selected examples of theoretical and experimental fitness landscapes. The decision graph illustrates the differences between fitness landscape models and provides examples of how they are used in the literature. Supplemental references (marked with S) are available in the supplemental information online. Abbreviations: BDM model, Bateson–Dobzhansky–Muller model; FGM, Fisher's geometric model; NA, not applicable; NK model, a model defined by the number of loci in the landscape, *N*, and the degree of epistasis between loci, *K*.

level because the relationship between phenotype and fitness is non-linear; the underlying fitness landscape is usually assumed to be a single-peaked Gaussian distribution. Martin et al. [40] showed that, although the epistasis between two random mutations is on average 0, epistasis between two beneficial mutations in FGM is on average negative. Gros et al. [41] extended this result by deriving an analytical expression of the mean epistasis between two random mutations, which can be positive, negative, or zero depending on the sharpness of the fitness peak in the extended FGM. These theoretical distributions of epistasis have been successfully matched with empirical data, both for pairs of random mutations and pairs of beneficial mutations [40].

In reality, mutations occur in genotype space rather than phenotype space. Theoretical work has recently shown that, although the FGM (despite the above-described pattern of negative epistasis) is relatively smooth in its phenotype–fitness map, the underlying genotype–fitness map can be highly epistatic [10,42]. Blanquart et al. [42] derived, under the assumption of FGM, that the amount of epistasis in an extracted genotype–fitness landscape depends on the choice of mutations. As predicted in this work, fitness landscapes tend to be smoother when built from mutations chosen from a realized adaptive walk (e.g., [12,43]) than those built from independent beneficial mutations that arose in the same reference background (e.g., [44]). A multitude of other experimental studies have reported ubiquitous epistasis in experimental fitness landscapes (e.g., [12,14,45–47]).

At the molecular level, epistasis occurs through interactions between proteins within a pathway (e.g., [45]) or between amino acids within a folded protein structure (e.g., [46]). In such cases



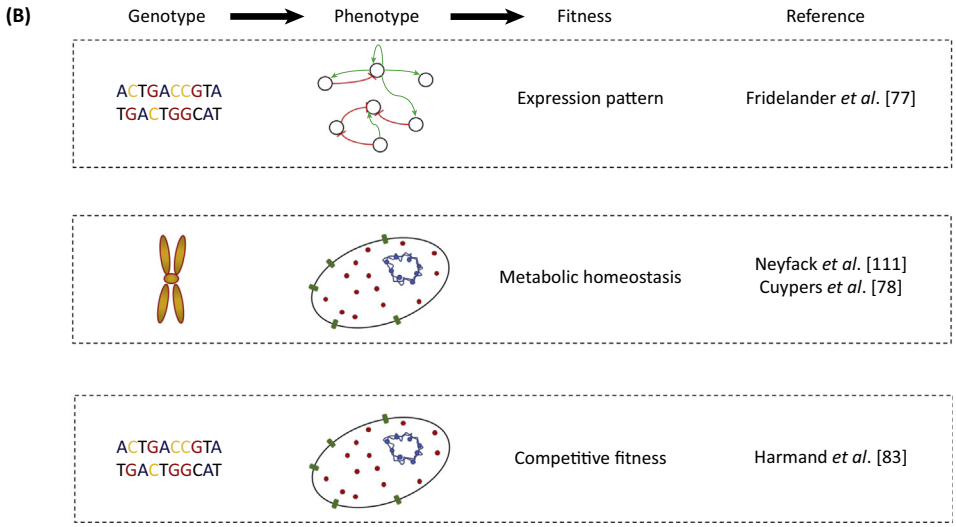


Fig. 2. Components and examples of genotype–phenotype–fitness maps. (A) Components of genotype-to-phenotype-to-fitness maps: such maps are normally degenerate because multiple genomic architectures give rise to fewer phenotypic

(Figure legend continued on the bottom of the next page.)

epistasis constrains how different subunits of a protein, a protein complex, or a pathway can coevolve, or how different rates of enzymatic activities need to match within a pathway [48]. The prevalence of epistasis within a biological network and its potential role in speciation was recently illustrated by Ono et al. [49]. From yeast populations adapted to a fungicide [50], the authors selected six first-step beneficial mutations within the ergosterol biosynthesis pathway, each mutation in a different gene. Consistent with the interaction of the genes in the pathway, they observed negative epistasis between the chosen mutations. This provides a biological explanation for the accumulation of BDM incompatibilities in isolated populations subject to identical environments. Mutations can speed up or slow down the production of ergosterol; hybrids that carry a mix of mutations from different populations can then randomly deviate from the optimum production speed and thus suffer from low fitness (also [51]). Surprisingly, the sign of the observed epistasis changed with an increasing concentration of the drug. In addition to linking biological networks and speciation, this study is noteworthy because it demonstrates the environment-dependence of epistasis and because the authors showed that considering the order of the genes in the pathway was not sufficient to explain the fitness of the double mutants. A mechanistic model based on fitness landscape theory could potentially provide additional power to explain the experimental results in the future.

Neutral evolution on fitness landscapes

Genetic drift of neutral mutations was proposed by Kimura as the main driver of evolution at the molecular level, whereas natural selection would mostly govern phenotypic evolution [52]. At first sight, this proposal is in stark contrast to models of genotype–fitness landscapes (e.g., NK, RMF [53,54]) in which no neutral mutations exist, such that evolution is governed by selection, often under the assumption that the effective population size is infinite ([3,55], also Box 3). We provide here various counter-examples in which fitness landscape models have been useful to understand how neutral evolution can aid both speciation and adaptation.

Specifically, the aforementioned concept of holey landscapes reconciles the neutral theory with the concept of fitness landscapes. Recall that holey landscapes are multidimensional genotype–fitness landscapes in which genotypes are either fit or lethal (Box 1). Schiffman and Ralph [56] showed recently that a holey-landscape-like architecture can emerge from complex molecular interactions that make up the cellular environment of an organism. The authors investigated theoretically how neutral changes could give rise to phenotypic divergence

outputs, which coalesce into some (single) measure of fitness (reviewed in [110]). Genes and their nucleotide sequences are taken as the fundamental unit of the genotype. These can be organized into groups of genes or gene clusters, and further into chromosomes and whole genomes. Likewise, gene products are considered to be the basic building block of the phenotype. The interaction of multiple gene products constitutes a biological network, and these can be used to map the physiology of a cell [78,111]. Fitness proxies are highly dependent on the biological level being probed. For example, structural stability (reviewed in [9,110]) or catalytic activity [47] are often considered as measures of fitness at the level of a single molecule, whereas more complex parameters, such as growth rate [47] are a frequently used measure of fitness in whole organisms. (B) Examples of genotype–phenotype–fitness maps. (Top) In this study [77] the consensus sequence of two copies of a transcription factor-coding gene, as well as their genomic binding sites, are represented by a sequence of nucleotides. The effect of the transcription factors on their downstream genes depends on the number of mismatches between the consensus sequence and the corresponding binding site. Fitness is measured relative to the expression level of the downstream genes. (Center) In these studies [78,111] a circular genome encodes the complete molecular machinery of a virtual cell. The phenotypic component is determined by a network of transcription factors, metabolic enzymes, and protein pumps that govern the uptake and processing of an external resource. The reproductive success of these virtual organisms depends on maintaining the internal concentration of the metabolized nutrient at a given value. (Bottom) In this study [83] a strain of *E. coli* was grown in different concentrations of nalidixic acid and the profile of antibiotic resistance was assessed relative to the presence or absence of mutations in the *gyrA* gene. The fitness of the mutants was measured in competition assays with a resistant strain and this was found to be consistent with an extended version of Fisher's geometric model (FGM).

and possibly hybrid incompatibility. In the model, the gene regulatory network of individual organisms is represented as a system of linear differential equations, whose output determines an oscillatory phenotype that in turn determines the fitness of an individual. The authors showed that speciation can occur without any direct selection because different and mutually exclusive regulatory solutions are reached through drift. Thus, their mechanistic model recapitulates previous results obtained for holey landscapes. Second, the accumulation of neutral standing genetic variation in a population as a result of plateaus in the fitness landscape can pave the way for innovation after a change in the environment. For example, Draghi et al. [57] simulated the evolution of self-replicating RNA molecules before and after an environmental shift. They found that standing genetic variation that was neutral in the initial background, but showed positive epistasis with subsequent mutations, facilitated the appearance of subsequent beneficial mutations by spreading the population on a fitness plateau. Such positively epistatic effects of neutral mutations were predicted for biological networks [58], and have been identified experimentally in viral proteins [59]. Thus, depending on the shape of the fitness landscape and how it changes across environments, neutral mutations can provide opportunities for adaptation.

Finally, rapid fluctuations in the environment might render evolution on rugged fitness landscapes effectively neutral if mutational effects change across environments. Such behavior can be captured by fitness seascapes, a concept developed to quantify adaptation in fluctuating environments [60]. Although fitness seascape models ignore neutral mutations, they might nevertheless converge to an effectively flat (static) fitness landscape when the environmental fluctuations are faster than the fixation time of mutations [61]. This points to an important open question. On the one hand, it is unknown how much fitness landscapes change across environments; on the other hand, we do not know how much natural environments fluctuate and, thus, how much commonly used fitness measures and the resulting experimental fitness landscapes indeed reflect reproductive success in natural populations.

Fitness landscapes as a framework to investigate the genotype–phenotype–fitness map

Genotype–phenotype–fitness models determine the effect of mutations at the phenotypic level and provide a more complete approach to studying fitness landscapes [62,63]. Although the

Box 2. Predicting outcomes of evolution using fitness landscape theory.

Fitness landscape models might allow us to predict evolution [5] through the analysis of adaptive walks and the estimation of the probability of the same outcome from different starting points on the landscape. An adaptive walk is the sequential fixation of beneficial mutations that take the population from its initial (low) fitness value to a fitness optimum [88–90]. Most models consider adaptive walks in asexual populations under a strong-selection weak-mutation regime, assuming that beneficial mutations are rare and, when they appear, become fixed [3,55,89]. Three types of adaptive walks have been defined (Figure II): (i) random adaptive walks [91], (ii) true adaptive walks [55,92], and (iii) greedy adaptive walks [88]. The type of walk considered determines the predictability of evolution on a given fitness landscape. Whereas from a given starting point populations will always reach the same adaptive peak in greedy walks, in random walks the probability of reaching the same peak in a rugged fitness landscape is low. For true adaptive walks, in which the next mutational step depends on the relative fitness advantage of all neighboring single-step mutants, the probability of reaching the same endpoint depends strongly on the structure of the landscape, in particular the need to transverse fitness plateaus will decrease said probability [3,88,89].

Unsurprisingly, the shape of the landscape also affects the predictability of evolution. The higher the number of local optima in the fitness landscape the smaller the probability that populations with different starting genotypes will reach the same optimum. The number of peaks in a rugged landscape increases with the number of loci and the prevalence of epistasis [93,94]. An increase in the number of peaks decreases the probability of reaching the global fitness optimum, but increases the accessibility of the landscape (i.e., that at least one monotonic path exists between a low fitness genotype and a fitness peak [95,96]). The accessibility of the fitness landscape also increases with the number of alleles per locus [97], for example when the amino acid level is considered instead of the nucleotide level [98]. So far it has been argued that phenotypic evolution is more easily predictable than genetic evolution [5,99]. By integrating expectations of epistasis across biological levels, genotype–fitness landscapes could develop from an abstract construct into an effective tool for the prediction of evolutionary paths in genotype space.

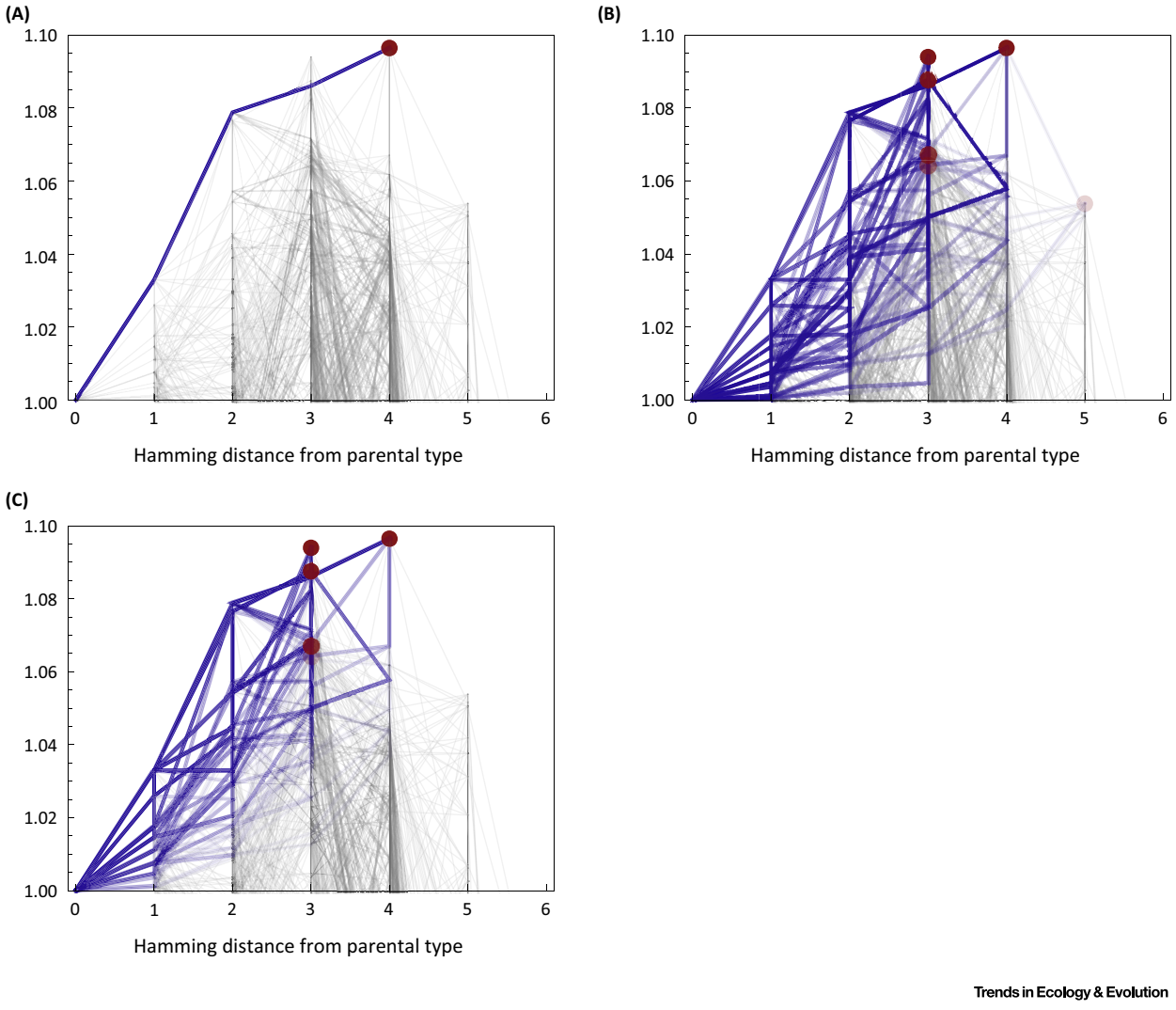


Figure II. Predictability of the different types of adaptive walks on a fitness landscape, demonstrated using data from [14]. In all panels, the *y* axis corresponds to fitness relative to the parental type and the *x* axis to the Hamming distance (the number of single mutational steps) from the parental type. In a greedy walk (A), the fittest single-step mutant neighbor is chosen deterministically. Thus different realizations starting from the ancestral type end up in the same fitness peak [88]. In a random walk (B), a new genotype is selected among the set of all fitter genotypes that are accessible by a single mutation [3,8]. This reduces the predictability of the adaptive walk. In a true adaptive walk (C), the new genotype is chosen with a probability proportional to the relative fitness advantage of all single-step mutant neighbors [3,55,88]. In this walk, large fitness differences between single-step neighbors lead to a higher predictability of the walk. Adaptive walks are represented by blue lines, and fitness peaks are marked as red circles. For each panel 500 simulations were performed starting from the ancestral type. Darker shades of blue and red indicate higher numbers of simulations using the path and ending in the fitness peak, respectively.

relationship between genotype and phenotype is complex [64], theoretical and empirical studies have pointed out several general properties that could be incorporated into generalized genotype–phenotype–fitness models. First, the dimensionality of the genotype space is larger than its phenotypic counterpart (genotype redundancy). This results in phenotypic robustness (many genotypes code for the same phenotype), which indicates that neutral evolution at the genotype level can play an important role in the genotype–phenotype–fitness map [6,62,63,65]. Second, experimental and theoretical studies found that a so-called genotype bias exists at the

Box 3. Wright's shifting balance versus the drift barrier – how population size tunes the shape of the fitness landscape.

The effective population size N_e determines the effectiveness of natural selection; mutations with a selection coefficient s in the interval $-1/2N_e < s < 1/2N_e$ behave indistinguishably from a neutral mutation [100]. Thus, differences in the population size affect evolution by altering the perceived local shape of the fitness landscape that a population is experiencing. On the one hand, a (temporal) reduction in the population size can make a landscape more connected, as proposed in Wright's shifting balance theory [1,101]. On the other hand, the population size limits the ability of the population to climb a fitness peak to the top, as illustrated in the drift–barrier hypothesis [102]. With smaller population size, larger plateaus of effectively neutral genotypes are created. In the limit of small population size, this results in a 'holey landscape' with only neutral and lethal genotypes.

The population size also directly affects two parameters that relate to adaptation dynamics on the fitness landscape: the rate of introduction of new mutations in a population increases with population size, whereas the probability of fixation of those mutations once introduced decreases [103]. In large populations, mutations that fix will segregate for a longer time before reaching fixation and, depending upon the mutation rate, can potentially encounter other segregating mutations. In asexual populations, this leads to clonal interference, which makes the fixation of mutations dependent on both their time of appearance and their selection coefficient, and which violates the strong-selection weak-mutation assumption that is the basis of many theoretical fitness landscape studies (Box 2). The complex aspects of the population size-dependence of adaptation dynamics on fitness landscapes have been studied both theoretically [48,104–106] and experimentally [107–109].

RNA and protein level [65–69], which means that the distribution of the number of genotypes per phenotype tends to be non-uniform, with the most frequent phenotypes being encoded by a large proportion of the genotypes. Although the field is far from developing a general approach that includes the described properties and constraints, we highlight here two types of promising studies that have combined theoretical and experimental work to give a better understanding of the relationship between genotype, phenotype, and fitness.

In molecular and systems biology, knowledge of biophysics coupled with high-throughput experimental methods has laid the foundation for linking theoretical to experimental fitness landscapes at the molecular and network level. For example, various genotype–phenotype–fitness maps have been created based on information from RNA or protein folding or from biological networks [62,63,70–72]. With respect to RNA or protein folding, the stability or binding specificity of a molecule can be predicted from the biophysical properties of nucleotide or amino acid sequences. These phenotypes can be interpreted as fitness proxies of the molecule, allowing the impact of different evolutionary forces (mainly selection and drift) on molecular evolution to be tested [9,70,73]. With respect to biological networks, the genotype–phenotype–fitness map arises from the interactions between network components, for example metabolic and gene-regulatory networks. These are usually defined by a set of nodes (e.g., genes or proteins, representing the genotype) and connections (e.g., activation or repression). The output (e.g., expression level) represents the phenotype, which can be mapped to fitness. Several studies have focused on investigating how the general properties of these networks evolve, such as the number of network components (complexity) [74–77] or the ability to generate novel phenotypes upon mutation (evolvability) [48,75,78]. For example, Friedlander et al. [77] modeled a system in which a transcription factor (TF) activates two downstream genes in response to two signals, and followed its evolution after a duplication event to investigate the conditions that favor specialization. In this model, the affinity of the TF to its DNA target is modeled according to biophysical principles and acts as a proxy for gene expression and fitness. The authors observed that, for one of the TFs, specificity to environmental cues evolves first while maintaining promiscuity in its binding-site affinity, whereas the second TF regulates only one gene, but is promiscuous in relation to the input signal. Thus, specialization proceeded

through small intermediate steps in which input signals are transmitted in a non-specialized manner to both TFs (crosstalk), with the interactions between TF-binding site specificity evolving in a neutral-like landscape for long periods of time, before specialization takes place. This work represents an accurate, but highly specific and parameter-heavy (bottom-up) genotype–phenotype–fitness map [75,79].

A complementary approach was taken by integrating the genotype–phenotype map into phenotype–fitness models such as FGM (introduced above in the section 'Epistasis in the Light of Fitness Landscapes'). FGM naturally incorporates several important features of the genotype–phenotype map, such as pleiotropy, epistasis, and the non-linear mapping between genotype, phenotype, and fitness. Owing to this flexibility, FGM has been used to study various evolutionary questions [3,8,10,18,40,79–81]. For example, FGM has been used to reproduce many patterns characteristic of speciation [81] and hybridization [82]. Interestingly, and in contrast to speciation models in which epistasis must be specifically defined (e.g., BDM), genetic incompatibilities and epistasis are emergent properties of FGM (see section 'Epistasis in the Light of Fitness Landscapes'). The blindness of the FGM to the underlying molecular features makes it readily applicable to experimental studies. Recently, Harmand et al. [83] combined experimental and theoretical approaches and explicitly included the genotype–phenotype map in FGM. The authors showed that, to describe the change of the fitness landscape across a gradient of antibiotic concentrations, it is necessary to take into account that mutations can impact differently on phenotypes across environments, and that changes in the environment might affect the height of the fitness peak and thus the rate of fitness increase. By introducing these theoretical considerations, they were able to draw a genotype–phenotype–fitness map that captured the specifics of antibiotic resistance evolution in *E. coli*. Thus, although the original FGM does not explicitly consider the genotype level, it provides a conceptually simple framework that naturally incorporates the genotype–phenotype map [8,10,18,42,79].

Another, less commonly used phenotype–fitness model is the multilinear approach that is derived from quantitative genetics. In this model, the genotype–phenotype map is directly modeled into the fitness landscape. The distribution of phenotypes in the population is described by the vector of mean phenotypes, and the *G* matrix, which is the variance–covariance matrix of the phenotypes. The mutational architecture of the phenotype distribution is described by the *M* matrix, which can take into account not only additivity but also epistatic interactions between loci [84]. This allows the evolutionary forces to act at different levels by shaping both the *G* and *M* matrices [84–86]. The multilinear approach is similar to FGM, but accommodates standing genetic variation that might exist in the population, and both recombination and mutation can contribute to changes in mean trait values [84,86]. Although more realistic, the multilinear approach comes with the caveat of being computationally heavy because it requires tracking of the *G* and *M* matrices.

In summary, much progress has been made theoretically and experimentally regarding the mapping of genotype to phenotype to fitness. Both bottom-up [62,63,70–72] and top-down [83] approaches have generated genotype–phenotype–fitness maps, and these have shown that integrating the genotype–phenotype relationship increases our ability to understand and predict evolution [72,87]. A major limitation of the described models is that they are either highly specific and heavily parameterized to describe a particular molecule or network, or that they do not consider mechanistic and molecular constraints. It will be a challenge to bridge this gap in future work, for example by incorporating biophysical constraints into FGM.

Concluding remarks

In the past 20 years the concept of fitness landscapes has developed from a purely theoretical and philosophical construct into an applied framework to analyze and interpret large datasets. Fitness landscape theory can be used to integrate the increasing amount of information on observed genome sequences, the biophysical properties of specific molecules, and the fitness of multiple organisms in various sets of conditions that we can now gather across the subfields of biology. Feeding this information into a single theoretical framework bridges the gap between evolutionary, molecular, and systems biology, and thus creates opportunities for interdisciplinary collaborative approaches to the study of evolution (see Outstanding questions).

In this review we show that, although at first sight fitness landscapes have been used to address apparently unrelated questions across fields, there are many commonalities between the different models and the emerging results. For example, scenarios of neutral evolution in biological networks often relate to the dynamics of evolution on a 'holey landscape' which was originally developed to describe speciation. Similarly, explicit genotype-fitness models such as the house-of-cards model converge to a 'holey landscape' in the limit of small population size. Explicit efforts to connect these different levels, for example by incorporating the genotype–phenotype map into FGM, have shown promising steps towards predicting patterns of evolution. Further combination of the different models in a unified framework will result in more realistic fitness landscape models that can be compared to experimental data. Integrated into a statistical framework, this approach could improve our understanding of the relative contributions of (intrinsic) molecular and (extrinsic) ecological constraints to adaptation and speciation, and, ultimately, improve our ability to predict evolution.

Outstanding questions

Properties of local fitness landscapes are often not informative of the shape of the global fitness landscape. Which properties of the global fitness landscape can be extrapolated from one or several local fitness landscapes? How is a local fitness landscape informative of its vicinity in genotype space?

How much does a fitness landscape change across an environmental gradient? Can this change (both in single-mutation effects and epistasis) be predicted using knowledge from molecular and systems biology?

How much do the various fitness proxies used in experimental studies matter for evolution in natural populations?

How can the accumulating mechanistic information on complex biological systems be integrated into a generalizable framework for the study of adaptation and speciation? Is there an optimal level of model complexity for predicting evolution?

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Appendix A Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.tree.2018.10.009.

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