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Recombination drives the evolution of mutational robustness

Sonia Singhal[1,*], **Shawn M. Gomez**[2,3,4], **Christina L. Burch**[1]

[1]Biology Department, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA.

[2]Curriculum in Bioinformatics and Computational Biology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27514.

[3]Department of Pharmacology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27514.

[4]Joint Department of Biomedical Engineering at University of North Carolina at Chapel Hill and North Carolina State University, Chapel Hill, NC, USA.

Abstract

Recombination can impose fitness costs as beneficial parental combinations of alleles are broken apart, a phenomenon known as recombination load. Computational models suggest that populations may evolve a reduced recombination load by reducing either the likelihood of recombination events (bring interacting loci in physical proximity) or the strength of interactions between loci (make loci more independent of one another). We review evidence for each of these possibilities and their consequences for the genotype-fitness relationship. In particular, we expect that reducing interaction strengths between loci will lead to genomes that are also robust to mutational perturbations, but reducing recombination rates alone will not. We note that both mechanisms most likely played a role in the evolution of extant populations, and that both can result in the frequently-observed pattern of physical linkage between interacting loci.

Introduction

An important property of any complex system of interacting parts is its robustness to perturbation. In biological systems, robustness to both genetic and environmental perturbations are manifest at every level of biological organization, from the organization of the genetic code to protein folding to developmental underpinnings of the phenotype [1]. Understanding the evolutionary origins of the robustness that characterizes biological systems has been a major goal of both systems and evolutionary biologists [2].

Although the evolution of robustness to environmental perturbation is well understood [3], the evolutionary origins of **genetic robustness** (Box 1), including robustness to recombination (**recombinational robustness**, the focus of this review) and mutation (mutational robustness), are less clear. Theory on the evolution of genetic robustness has

*Corresponding author. Address: Biology Department, UNC Chapel Hill, CB#3280, Chapel Hill, NC, 27599. ssinghal@email.unc.edu.

almost exclusively considered the ability of selection to favor higher robustness in populations that exist in the vicinity of a single fitness optimum. We can envision selection for robustness through the analogy of a **fitness landscape** (Box 1). Figure 1a depicts a simple 2-locus landscape. In this model, it is possible to mutate from allele *a* to allele *A* and from allele *b* to allele *B*, giving four total genotypes (*ab, aB, Ab,* and *AB*) of varying fitness. In Figure 1a, it is possible to reach the highest-fitness genotype (*AB*, a fitness **peak**) from the lowest-fitness one (*ab*) through mutations that increase fitness monotonically. We consider such a fitness landscape to be **smooth**. In contrast, in Figure 1b, the effect of a mutation from *a* to *A* depends on the identity of the allele at the second locus (*b* or *B,* an example of **sign epistasis**; Box 1). The resulting fitness landscape has two fitness peaks (genotypes *ab* and *AB*), and we consider it to be **rugged** (Box 1). If the *aB* and *Ab* hybrids are deleterious, we call the collection of *AB* or *ab* alleles a **coadapted gene complex** (Box 1).

On smooth fitness landscapes, genetic robustness is achieved by movement of the population from a steeper region of the landscape, where mutations have large effects, to a flatter “plateau” region, where mutations have smaller effects. In this scenario, genetic robustness is expected to evolve in populations with unusually high mutation rates as a direct response to selection to minimize the mutation load [6–8]. However, because mutation load generally exerts only a small selective pressure on a population [1], the genetic robustness that characterizes biological systems is thought to have evolved not as a direct response to selection, but as a side effect of selection for another property (e.g., environmental robustness) [2,9].

This view depends critically on the assumption that populations exist on smooth fitness landscapes. By contrast, recent technological advances in genetics and genomics have revealed that both laboratory and natural populations exist in rugged (multi-peaked) regions of the fitness landscape. Two lines of evidence support this conclusion. First, different populations can evolve to occupy different local optima, each of which corresponds to a distinct genotype. Perturbing these optimal genotypes, such as through recombination, can lead to fitness decreases. In eukaryotes, F2 crosses of parents from different populations frequently show reduced fitness, contributing to reproductive isolation between these populations [10–13]. In laboratory experimental evolution, genetic incompatibilities are common between alleles fixed in different laboratory populations [14,15]. Second, individuals within a single population can occupy different local fitness optima. In the laboratory, crosses or mixed populations of different recombinant inbred lines result in an underrepresentation of particular allele combinations (in *Drosophila* and *Arabidopsis,* [16], in mice, [17,18]), indicating the presence of low-fitness valley genotypes between higher-fitness parents. The presence of incompatible alleles shared among recombinant inbred lineages further suggests that these alleles segregate at polymorphic frequencies in natural populations [16].

The examples above highlight that, in rugged fitness landscapes, recombination can be a deleterious perturbation to the genome. The average magnitude of the fitness decrease between recombinant offspring and their parents is referred to as **recombination load** (Box 1). The presence of recombination load suggests that there may be selection for parents

whose recombinant offspring maintain high fitness. Furthermore, recent *in silico* evolution experiments have demonstrated that recombination can select for robustness to mutations, even in conditions where mutation alone cannot [19–22]. Critically, although these experiments differed in the details of their system (biological or engineered) and implementation, they shared the characteristic that their component parts interacted to determine fitness, resulting in a rugged fitness landscape. These data suggest that the recombination load experienced by sexually reproducing populations can play a critical role in the evolution of mutational robustness by imposing selection for movement of the population away from more rugged regions of the fitness landscape and into smoother regions of smaller mutational effect.

In the sections that follow we describe two mechanisms by which *in silico* populations have been observed to evolve a reduced cost of recombination: by altering a physical property of the genome to reduce the rate of recombination between loci that comprise coadapted gene complexes (**increasing physical linkage**); or by decreasing the number of costly interactions between loci that experience high rates of recombination (**decreasing epistasis**). We discuss biological advantages of these mechanisms and their evolutionary consequences on the genome. Importantly, decreasing epistasis between loci is expected to result in mutational robustness, but increasing physical linkage is not. Finally, we note that in real biological systems, genes that interact to form coadapted gene complexes commonly exist in close proximity on the genome, a pattern that could have resulted from either of these mechanisms (Box 1).

Reduction of recombination load by increasing physical linkage

As early as Fisher [23], mechanisms that favor the reduction of recombination between coadapted gene complexes have been expected to be selectively favorable, because such mechanisms reduce recombination load. One such mechanism is to decrease the physical distance between interacting genes (transition from Figure 2a to Figure 2b). Physical proximity of loci lowers the likelihood that the alleles are broken apart by recombination; they are instead inherited as a single unit. The consequences of this solution for mean population fitness are illustrated graphically in Figure 1e: Because the alleles become linked, the population can transit from *ab* to *AB* without crossing a fitness valley. Alleles that do not contribute to a coadapted gene complex, in contrast, are expected to make only small contributions to recombination load, and selection for reduced recombination (e.g., via physical proximity) between such loci is expected to be weak or absent. The difference in the strength of selection against recombination at interacting loci compared to independent loci results in a genome in which interacting loci are more likely than are independent loci to evolve to become physically proximal.

The evolution of **increased physical linkage** as a consequence of recombination has been demonstrated in computational models. Yang *et al.* [24] performed evolutionary simulations on a model of linear chromosomes with loci separated by a variable distance. Loci in their model could interact positively (*AB* double mutant occurred more frequently than expected from a multiplicative model) or negatively (*AB* double mutant occurred less frequently than expected from a multiplicative model), or not interact. For loci exhibiting positive epistasis,

a reduction in distance between the loci was advantageous in recombining populations. Furthermore, the magnitude of the reduction in distance correlated with the magnitude of the epistatic effect: Lower distances were more favorable for loci with strong than weak positive epistasis. Misevic *et al.* [20] similarly found that recombination affected genome organization in a different computational system of self-replicating, evolving computer programs (AVIDA). In their study, populations that experienced homologous recombination evolved genomes in which loci that contributed to the same function (one of nine mathematical operations) tended to be physically proximal. These loci occupied a highly compact space in the genome and reduced their spatial overlap with loci that contributed to different functions. Populations that reproduced asexually, in contrast, did not evolve the same degree of linkage among loci that contributed to the same function [20].

Biologically, physical linkage between genes may be favorable not only because of coinheritance of coadapted genes, but also because it allows co-expression [25]. Co-expression and co-inheritance may be particularly vital if the gene products result in toxic intermediates [26,27] or are themselves toxic. For example, toxin/antitoxin systems in microbes involve toxin genes that are physically linked to their corresponding antitoxin gene (in bacteria, [28]; in yeast, [29]). Recombination that results in non-corresponding toxin and antitoxin alleles is likely to produce offspring with reduced or no viability. A *Schizosaccharomyces* meiotic drive system, in fact, even encodes the toxin and antitoxin on overlapping transcripts [29].

In less lethal examples, physical proximity of interacting loci is presumed to play a role in the maintenance of operons and gene clusters [25]. Essential gene clusters in yeast are typically found in recombination cold spots [30], suggesting that the maintenance of vital interactions between genes in the cluster is selectively favored. Physical linkage of the six genes in the *DAL* cluster in *Saccharomyces cerevisiae* may have been selectively favored because alleles at each locus only interacted well with specific alleles at other loci in the cluster [26]. Similarly, chromosomal inversions, which reduce the likelihood of recombination by reducing homology between chromosomes, have been implicated in the maintenance of species boundaries [31–33], mimicry in butterflies [34,35], and hierarchical social structure in fire ants [36].

Reduction of recombination load through changes in epistasis

Particularly in cases where physical linkage of interacting alleles is not possible, populations may instead evolve a lower cost of recombination by **decreasing epistasis** (reducing ruggedness) between loci (transition from Figure 2a to 2c). That epistasis can evolve is not a new idea: Multiple studies document that the strength and/or direction of epistatic effects between focal loci can be altered by a higher mutation rate [6–8], an environmental change [40,43–45], the variant at non-focal loci [37,40,42,46,47], or the relative position of the loci in a signalling cascade [48]. We posit that, in the face of a rugged fitness landscape, recombination should impose particularly strong selection to reduce the number of loci that interact as part of coadapted gene complexes, leading to a reduction in sign epistasis, particularly between distal genes.

Although few studies have systematically compared ruggedness between physically proximate and distant loci, we can consider an extreme case where loci within a gene are physically proximate and loci in different genes are physically distant. For example, Poon *et al.* [41] examined the locations of second-site suppressor mutations, which compensate for the effects of highly deleterious or lethal mutations, in 23 organisms from different taxa. Of 1021 distinct suppressor mutations, 79% occurred in the same gene as the deleterious mutation they compensated, suggesting stronger within-gene than between-gene interactions. Similarly, Sackman and Rokyta [42] engineered 60 pairwise combinations of 15 beneficial mutations that arose in independent laboratory-evolved populations of the bacteriophage ID8. Importantly, the combined effects of the 15 mutations were unknown, eliminating a likely source of bias [15]. Sackman and Rokyta found more instances of epistasis (including cases of lethality) between mutations in the same gene than between mutations in different genes [42]. However, we note that it is unclear whether the physical distance between loci affects the strength of epistasis beyond their organization into genes. More data are needed to determine whether the epistasis between genes depends on the physical distance between them.

Computational studies indicate that epistasis can evolve in recombining populations. *In silico*, populations that recombine evolve to exhibit less sign epistasis between segregating alleles, and, as a result, higher recombinational robustness [19,21,22,52] than populations that do not recombine. The mechanism stems from the different kinds of alleles capable of fixing in asexual or recombining populations. In asexual populations, beneficial (or deleterious) mutations experience selection in the particular genetic background in which they arose, and selection includes epistatic effects with other alleles in that background [53–55]. Thus, in an asexual population, a new mutation that is beneficial in the genetic background in which it arose can achieve fixation even if it is deleterious in every other genetic background in the population. On the other hand, in a recombining population, the probability that a new mutation achieves fixation depends not (only) on its fitness effect in the genetic background in which it arose, but on its marginal (average) effect across all genetic backgrounds in the population [55,56]. Over time, alleles fix in recombining populations specifically because they did not experience strong sign epistasis with the variation segregating at other loci in the genome. In this way, recombinational robustness evolves as a distributed property of the genomes in recombining populations.

Importantly, the evolution of recombinational robustness in the computational models was accompanied by mutational robustness: Mutational effects on fitness were on average smaller in magnitude in evolved recombining populations than in evolved asexual ones [19,20,22,52], indicating a smoother underlying fitness landscape. We present an example of this type of landscape change in Figure 1. Recall that in Figure 1b, the A and B loci exhibit sign epistasis, so that the sign of the fitness effect of a mutation from a to A, positive or negative, depends on the identity of the allele at the B locus (b or B). In our specific example, genotypes Ab and aB contribute to recombination load. Now, suppose the A and B loci occur in a genome that contains a locus C that modifies the epistasis between them, such that a transition from allele c to allele C moves the population from the rugged region of the landscape in Figure 1b to the smooth region in Figure 1a. A population adapting from a starting genotype aBc might progress through a state in which the locally adaptive genotypes

ABc and *abc* were both segregating at moderate frequencies in the population. As can be seen from a comparison of Figures 1c and 1d, the mutation from *c* to *C* becomes adaptive in this state: The allele *C* has a higher marginal (average) fitness across all the genetic backgrounds in the population. When *C* fixes, the sign epistasis between loci *A* and *B* is eliminated, the local landscape becomes less rugged, and recombination load decreases. As a result, both recombinational and mutational robustness increase. (Our example here involves an increase in the fitness of genotypes *Ab* and *aB.* A modifier *C* that decreased the fitness of genotypes *ab* and *AB* could also fix, as long as its marginal fitness was higher than that of allele *c*.)

Under the hypothesis that recombination selects for **decreasing epistasis** (i.e. reduced ruggedness), we would expect that the fitness landscapes of organisms that recombine frequently would be less characterized by sign epistasis between loci than those of asexually reproducing organisms. Although systematic comparisons of fitness landscapes in recombining and asexual populations have not been done, prior measurements provide some data consistent with this hypothesis. Asexual populations often fix "cohorts" of mutations, suggesting either epistatic interactions between those mutations or the fixation of linked mutations [53,54,57,58]. Direct measurement of fitness landscapes of asexual organisms typically reveals sign epistasis [59,60]. Simulations of sexual reproduction on a fitness landscape of the asexually reproducing *Aspergillus,* in fact, appeared to disfavor recombination [60], indicating a high recombination load. On the other hand, fitness landscapes of organisms that regularly recombine exhibit less sign epistasis [61,62]. Further characterization of fitness landscapes across taxa is necessary, but reproductive mode may explain discrepancies in the amount of epistasis measured in empirical fitness landscapes.

Future directions

It is clear from *in silico* evolution experiments that decreasing epistasis between loci, in addition to increasing physical linkage, can make a substantial contribution to the evolution of mutational robustness. Recombination load due to physically distant interacting loci (Figure 2a) may be resolved either by bringing the loci into closer proximity (transition from Figure 2a to 2b) or by reducing the epistatic interactions between them (transition from Figure 2a to 2c). However, it is difficult to determine how each mechanism contributes to genomic structure in extant natural populations, because both mechanisms are expected to produce a genomic pattern in which the genes that interact (i.e., as coadapted gene complexes) exist in close physical proximity (Figure 2b). This pattern could evolve due to changes in linkage, epistasis, or both. Moreover, extant recombining populations can experience more complex changes in linkage and epistasis. For example, clusters of linked genes involved in the *DAL* gene cluster in *S. cerevisiae* [26] and the oat avenacin pathway [51] include one (oat avenacin) or more (*DAL* gene cluster) genes whose ancestral homologs are unlinked from the cluster. This suggests that these gene clusters were formed, at least in part, when non-homologous recombination brought genes in the cluster into physical proximity (transition from Figure 2c to 2b). In the absence of an intermediate (either Figure 2a or 2d), it is unclear whether interactions between genes in the cluster arose prior to their becoming linked (Figure 2a), or if their proximity after becoming linked allowed formation of new interactions (Figure 2d). Comparative studies that identify differences in both the

number and genomic position of genes within coadapted gene complexes are needed to distinguish which transition is more likely to result in the observed pattern (Figure 2b). A phylogenetic signal that selection acts to decrease the epistasis that contributes to recombination load may be that genes homologous across taxa are more likely to interact when they are found in proximity on the genome, but not when they are distributed.

We also note the importance of multi-locus fitness landscapes in understanding the consequences of recombination on the genome. If recombination drives the evolution of mutational robustness, then we predict that recombining populations will exhibit less ruggedness between loci than asexual populations. Empirical fitness landscapes are typically based on mutations within a single gene; less often do they include epistatic interactions between two or more genes. However, determining exactly how rugged natural fitness landscapes are—and thus how much recombination load populations are likely to experience—requires evaluation of between-gene epistasis. Libraries of double knockout mutants, such as the one in [63], can be used to examine high-level patterns of epistasis between entire genes across the genome. To quantify how recombination shapes fitness landscapes, similar data will be necessary from both recombining and non-recombining populations.

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Box 1.

Glossary of terms.

Genetic robustness. A property of a lineage in which perturbation of the genome, by mechanisms such as recombination or mutation, results in offspring with similar fitness to their non-perturbed parent. In this review, we consider one particular mechanism of genetic robustness: that in which genetic robustness emerges as a distributed property of a genome, reducing the average effect of deleterious perturbations. Whether this mechanism is expected to reduce [4] or increase [5] the effects or frequency of beneficial perturbations is beyond the scope of this review.

Recombinational robustness. A property of genetically variable populations in which recombination of the parental genome tends to result in offspring with similar fitness to the parent.

Fitness landscape. A simplified, 3-dimensional visualization of the relationship between genotype and fitness. Each genotype lies in the horizontal plane, and adjacent genotypes are single-step mutational neighbors that differ from one another in one allele at one locus. The corresponding fitness of these genotypes is displayed in the vertical plane. See Figure 1 for a two-locus example. High-fitness genotypes whose single-step mutational neighbors have lower fitness occupy a local fitness **peak** or fitness optimum. Low-fitness genotypes whose single-step mutational neighbors have higher fitness occupy a local fitness **valley** or fitness minimum. Populations move through the landscape as mutations alter their genotype and are selected based on the fitness of those genotypes. In an adaptive process, the population will move uphill, from genotypes of low fitness to a local fitness peak (e.g., genotype AB in Figure 1a).

Ruggedness. An indication of the number of local peaks on a fitness landscape. A landscape with only one peak is considered to be **smooth**, and the highest-fitness genotype can be reached from any other genotype through single-step mutations. In contrast, an increase in the number of peaks indicates multiple local fitness optima. Populations that attain a local optimum may not be able to attain the global optimum through single-step mutations without crossing through a low-fitness valley. Although the 3-dimensional cartoon in Figure 1 is a simplification, real, high-dimensional landscapes will also be characterized by regions that differ in ruggedness.

Epistasis. A situation where the fitness effects of loci depend on the genetic background in which they occur. Epistasis is typically measured as a departure from a null hypothesis of additivity (or multiplicativity) of the individual effects of the alleles at each locus, and represents an interaction between loci. Epistasis can result from a difference in the expected magnitude of fitness effects, a difference in the direction of the effects, or both.

Sign epistasis. An epistatic interaction that changes the direction of the fitness effect with respect to the individual alleles; for example, two individually beneficial alleles that are deleterious in combination, or two individually deleterious alleles that are beneficial in combination. In a fitness landscape, sign epistasis results in ruggedness.

Coadapted gene complex. Sets of loci are referred to as coadapted gene complexes when there exist alternative high fitness combinations of alleles at those loci (e.g. *AB* and *ab* in Figure 1b), either segregating within the same population or fixed in different populations, such that substituting the alternative allele at any of the loci in the complex (e.g. *Ab* or *aB* in Figure 1b) would be deleterious. The alternative high fitness combinations correspond to different fitness peaks on a rugged landscape.

Recombination load. A measurement of the extent to which recombinant offspring have reduced fitness with respect to their parents, due to the breakup of favorable parental allele combinations (i.e., of coadapted gene complexes). For the purposes of this review, a low recombination load indicates recombinational robustness.

Increasing physical linkage as a mechanism of recombinational robustness. Modulation of recombination load based on the physical distance between loci. Under this mechanism, recombinational robustness increases by reducing the physical distance between loci that comprise individual coadapted gene complexes. In genomes that have evolved robustness by this mechanism, the loci that comprise individual coadapted gene complexes will exist in close physical proximity.

Decreasing epistasis as a mechanism of recombinational robustness. Modulation of recombination load based on the strength of interactions between loci. Under this mechanism, recombinational robustness increases by reducing the sign epistasis between loci that interact as components of coadapted gene complexes. The strength of selection to reduce sign epistasis increases with the probability of recombination and, therefore, the physical distance between the interacting loci. In genomes that have evolved robustness by decreasing epistasis, coadapted gene complexes will be comprised only of loci that were originally in close physical proximity. Note that both mechanisms of robustness, increased physical linkage and decreased epistasis, result in the same genomic pattern in which interacting loci exist in close physical proximity.

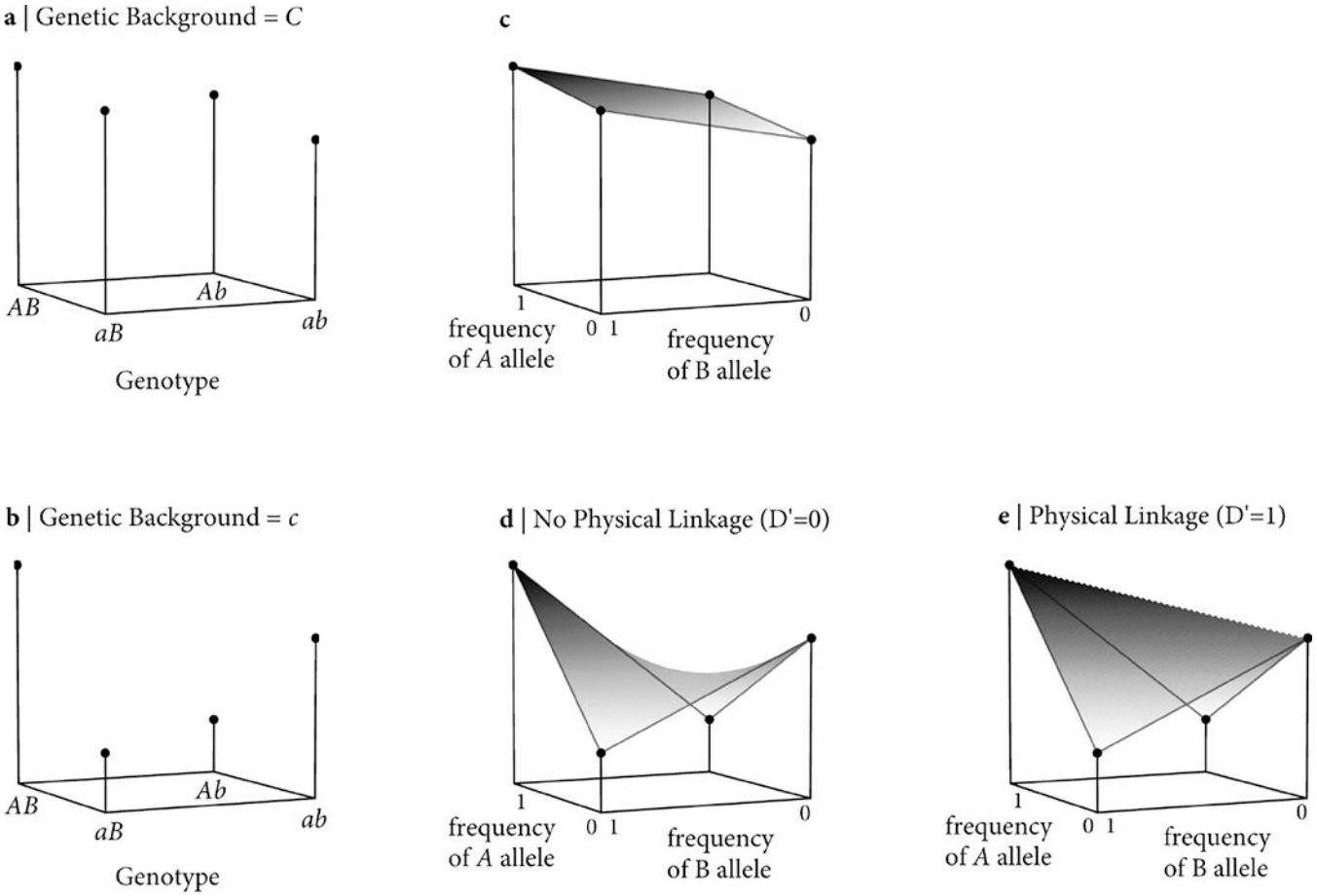

Figure 1.

Fitness landscapes for haploid genomes comprising 3 biallelic loci. On the left, genotypic fitness landscapes depict the fitnesses of the haploid *ab*, *Ab*, *aB*, and *AB* genotypes in genetic backgrounds containing (**a**) allele *C* or (**b**) allele *c* at a third locus. In both genotypic landscapes, sequence space is represented in the lower horizontal plane and the fitness value of each genotype is projected upward. Landscapes on the right show population mean fitness as a function of allele frequencies. The additive genotypic fitness values in panel **a** yield the smooth population mean fitness landscape in panel **c**, whether or not there is linkage disequilibrium between the *A* and *B* loci. The sign epistasis in panel **b** yields the rugged landscape in panel **d** when the *A* and *B* loci are in linkage equilibrium (D' = 0), but can yield the smooth landscape in panel **e** if the *A* and *B* loci are in linkage disequilibrium (D'=1, e.g., as a result of close physical proximity).

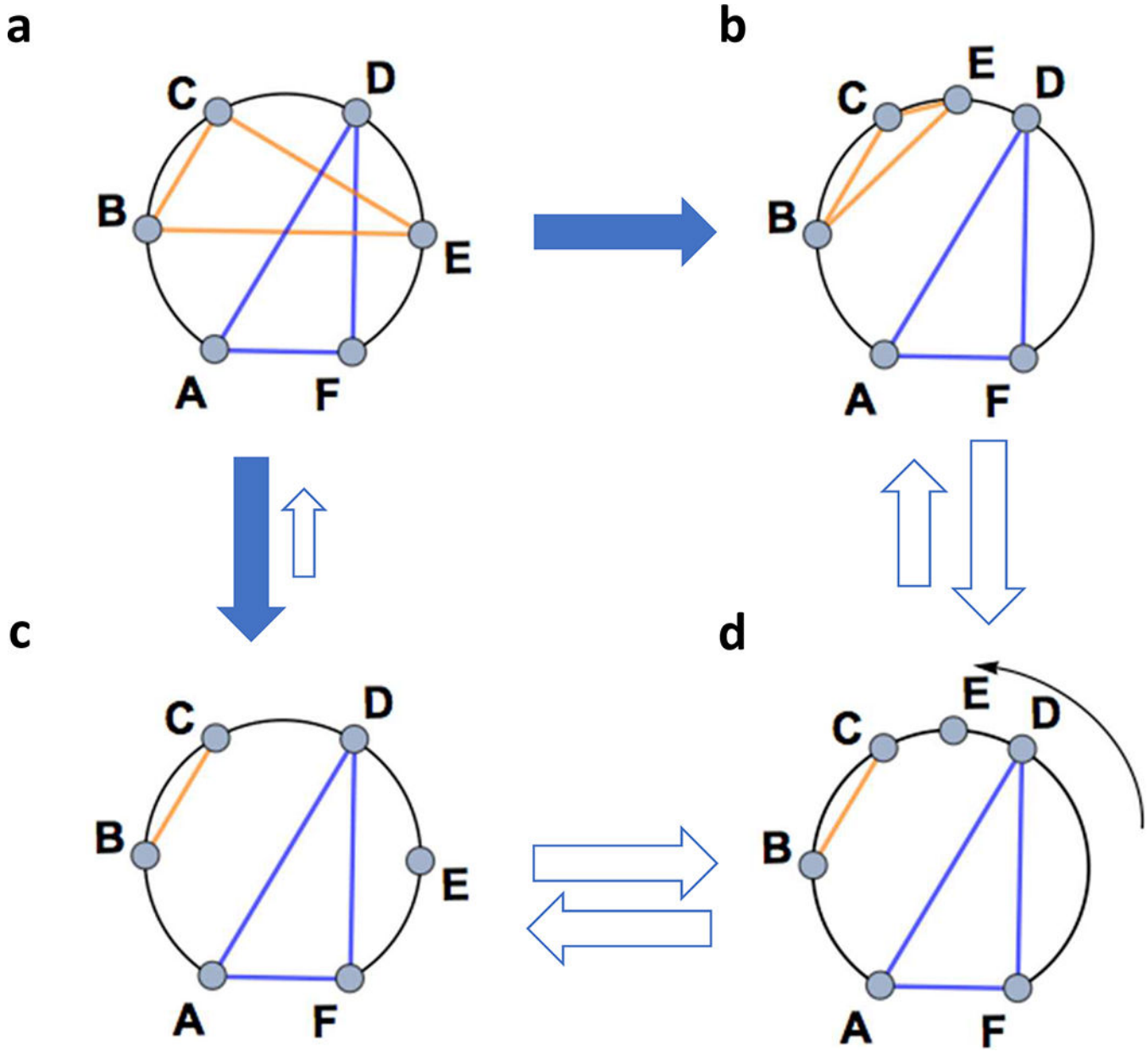

Figure 2.

Changes in physical linkage and epistasis. Each black arc represents a contiguous, linear genomic segment; blue and orange lines within the arc represent interacting loci; and the relative sizes of the block arrows indicate the relative strength of selection due to recombination load. The distributed pattern of epistasis in panel **a** is expected to result in a high recombination load, which may be resolved either by increasing physical linkage between loci *B, C,* and *E* (**b**); or by reducing epistasis (**c**). Both mechanisms have been observed in computational models (filled arrows) and result in a genomic pattern in which interacting loci exist in physical proximity. From the genome in panel **c**, an interaction

between loci *B, C,* and distal locus *E* could evolve without a large increase in recombination load if locus *E* moved into proximity with loci *B* and *C* (**d**).