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Epistasis between deleterious mutations and the evolution of recombination

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Epistasis and the evolution of recombination are closely intertwined: epistasis generates linkage disequilibria (i.e. statistical associations between alleles), whereas recombination breaks them up. The mutational deterministic hypothesis (MDH) states that high recombination rates are maintained because the breaking up of linkage disequilibria generated by negative epistasis enables more efficient purging of deleterious mutations. However, recent theoretical and experimental work challenges the MDH. Experimental evidence suggests that negative epistasis, required by the MDH, is relatively uncommon. On the theoretical side, population genetic models suggest that, compared with the combined effects of drift and selection, epistasis generates a negligible amount of linkage disequilibria. Here, we assess these criticisms and discuss to what extent they invalidate the MDH as an explanation for the evolution of recombination.

Epistasis and the evolution of recombination

Frequently, an organism having a certain combination of alleles at different loci will have a higher or a lower fitness than would be expected given the average fitness effects of each single allele. This implies that the fitness of any specific genotype results not only from the independent selective effects of alleles at different loci, but also from interactions between the alleles at these loci. These interactions are termed 'epistasis'. In the simplest case (two loci with two alleles each), epistasis can be quantified by comparing the fitness of the double mutant and the wild type (the extreme genotypes) with the fitness of the single mutants (the intermediate genotypes) (see Glossary). Epistasis is positive or negative when the extreme genotypes have a higher or lower fitness, respectively, than would be expected from the fitness values of the intermediate genotypes. The strength and sign of epistasis have important roles in biology [1], from applied aspects of quantitative genetics (e.g. animal and plant breeding) to fundamental problems in evolutionary biology (e.g. speciation, mutational load and the maintenance of genetic diversity). Within evolutionary biology, one of the most hotly debated roles of epistasis is its relevance to the evolutionary maintenance of sexual reproduction and recombination. It is this role of epistasis that is the focus of this review.

Genetically, the only effect of recombination is to shuffle combinations of alleles. Such shuffling only has an effect on the frequency of genotypes in the population if the alleles are in linkage disequilibrium (LD), that is if there are statistical associations between alleles at different loci. Thus, any genetic theory explaining the maintenance of recombination must show why breaking down LD is beneficial. Epistasis is one force that can generate LD: if a combination of two alleles at different loci is fitter than expected (based on their individual effects), then this combination will be found more frequently than predicted from the allele-frequencies. An overrepresentation of the extreme or intermediate genotypes corresponds to positive or negative LD, respectively. If no other forces generate LD, positive epistasis generates positive LD, and negative epistasis generates negative LD [2].

Deleterious mutations and recombination

Several theories have been proposed that specify conditions under which breaking up LD generated by epistasis is

Glossary

Epistasis: quantifies the interaction between alleles at different loci. For the evolution of recombination, the relevant measure is how much the combined effect of a set of alleles on fitness differs from the product of the effects of the individual alleles in isolation. In a haploid two-locus–two-allele model with haplotypes *ab*, *Ab*, *aB*, *AB* and fitnesses w_{ab}, w_{Ab}, w_{aB}, w_{AB}, epistasis is defined as $e = w_{ab}\,w_{AB} - w_{Ab}\,w_{aB}$. For positive epistasis, the extreme genotypes *ab* and *AB* (wild type and double mutant) are fitter than expected from the intermediate haplotypes *Ab* and *aB* (single mutants) on a multiplicative scale. Conversely, for negative epistasis the extreme genotypes are less fit than expected (Box 1).

Linkage disequilibrium (LD): quantifies the statistical association between alleles at different loci. Specifically, it measures the deviation of the frequency of a combination of alleles from the genotype frequency expected from the frequency of the individual alleles. Mathematically, the LD between alleles at two loci is defined as $D = f_{ab} - f_a\,f_b$, where f_a is the frequency of individuals having allele *a* at locus 1, f_b is the frequency of individuals having allele *b* at locus 2, and f_{ab} is the frequency of individuals having allele *a* at locus 1 and allele *b* at locus 2. An equivalent definition (for a biallelic pair of loci) is $D = f_{ab}\,f_{AB} - f_{Ab}\,f_{aB}$. A positive (negative) LD implies that the combination of both alleles is more (less) frequent than expected by the frequencies of the individual alleles.

Mutational-deterministic hypothesis (MDH): the MDH posits that genetic shuffling has evolved as a means to purge deleterious mutations. Specifically, the MDH assumes that interactions between deleterious mutations predominantly exhibit negative epistasis and that the negative LD (induced by this epistasis) is the primary reason why recombination improves the purging of deleterious mutations and is thus selected for.

Physiological epistasis: the distribution of epistatic interactions among randomly introduced mutations independent of their frequency of occurrence in natural populations.

Population epistasis: the distribution of epistatic interactions that occur in a natural population weighted by their occurrence.

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beneficial (Box 1). One of the most prominent of these is the mutational deterministic hypothesis (MDH) [3–6], which postulates that the main advantage of recombination is that it helps in purging deleterious mutations from a population by breaking down LD created through epistatic effects. Importantly, the hypothesis is based on the assumption that deleterious mutations tend to exhibit negative epistasis (i.e. deleterious mutations tend to reduce fitness more strongly when combined than would be predicted by their mean individual effects). The appeal of this hypothesis is that it could explain the ubiquity of sexual reproduction, because it is likely that deleterious mutations are a strong selective force in most organisms. (Although the MDH focuses on deleterious mutations, an analogous argument has been made for beneficial mutations with negative epistasis [7]; Box 1).

The MDH originally proposed negative epistasis as an explanation for the maintenance of sexual reproduction in a population challenged by an asexual invader [4,6]. However, if negative epistasis is to explain the ubiquity of genetic shuffling in general, then two distinct questions that must be addressed:

First, what is the advantage of sexual compared to asexual reproduction? Most multicellular eukaryotes

Box 1. The role of epistasis for the evolution of recombination

From the population genetic perspective, the only effect of recombination is to break down LD. Thus, epistasis results in selection for higher (lower) recombination when it is beneficial (detrimental) to break down LD generated by epistasis [12]. Such a benefit or disadvantage can be realized in two ways, either as a long-term or a short-term effect.

Long-term effect

Epistasis generates LD of its own sign. For example, negative epistasis means that the extreme genotypes (the wild type and the double mutant) are less fit than expected and, thus, they tend to be underrepresented (negative LD). Breaking down negative LD reduces the frequency of the overrepresented class (the single-mutant genotypes) and increases the frequency of the underrepresented class (the wild type and double-mutant genotypes) (Figure Ia). This increase in frequency of extreme genotypes (and decrease of intermediate genotypes) increases the variance in fitness in the population and, therefore, the response to selection (Figure Ib). This increased response can be beneficial for two reasons: it enables either (i) a more efficient purging of deleterious mutations (the MDH); or (ii) a faster response to directional selection (e.g. in new environments). Importantly, these long-term benefits depend on a predominance of negative LD (which could be generated by negative epistasis); in the opposite case (positive LD), breaking down LD slows down the response to selection.

Short-term effect

Assume, for simplicity, that epistasis is negative and LD is positive (the reasoning is identical for the opposite case). This means that the extreme genotypes are less fit than expected (negative epistasis) but overrepresented (positive LD), whereas the intermediate genotypes are more fit than expected but underrepresented. Because recombination shifts genotypes from the overrepresented class to the underrepresented class, it increases the number of the fitter-than-expected but underrepresented intermediate genotypes and decreases the number of the less-fit-than-expected but overrepresented extreme genotypes. Thus, if LD and epistasis have opposite signs, there is a short-term benefit, because recombination increases the mean fitness of the offspring relative to their parents. Conversely, if epistasis and LD have the same sign, recombination lowers the mean fitness of the offspring (short-term disadvantage). Because epistasis tends to generate LD of its own sign, the short-term effect tends to favor lower recombination rates. (One way in which epistasis and LD can have different signs is under fluctuating epistasis, as is the case, for example, in the Red Queen Hypothesis [38]).

Following Ref. [39], we illustrate, in Figure Ic, the combined long-term and short-term effect as a function of LD and epistasis.

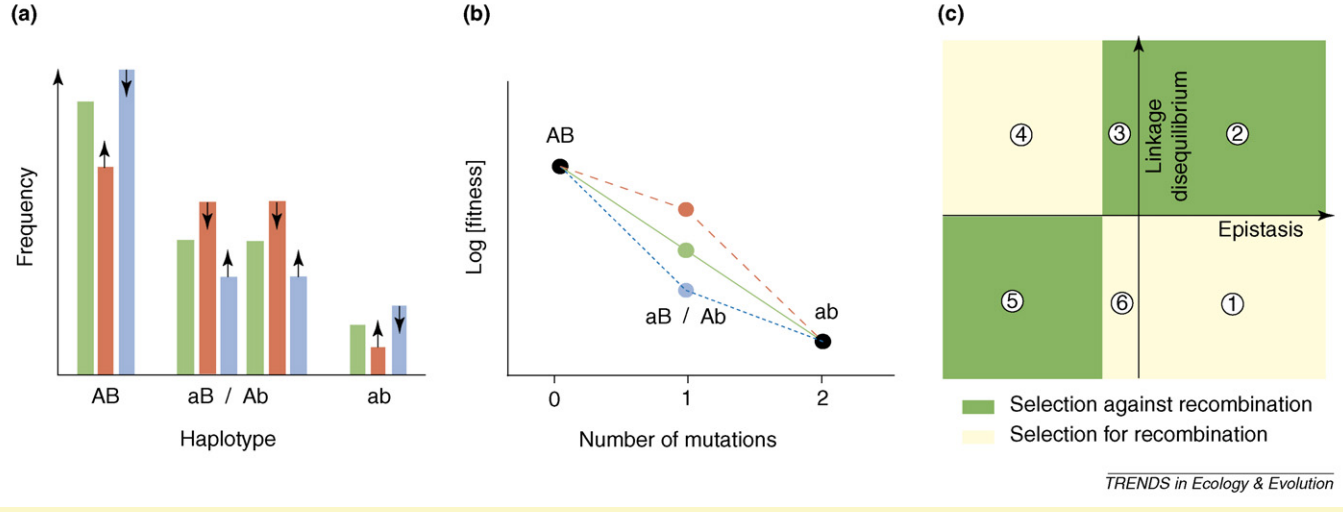

Figure I. LD and epistasis. **(a)** the effect of recombination on LD. When there is no departure from non-random associations between alleles, the haplotypes are in LD (green bars). Negative (positive) LD corresponds to an overrepresentation (underrepresentation) of the intermediate haplotypes, *aB* and *Ab*, compared to the extreme haplotypes, *AB* and *ab*, (red and blue bars, respectively). Recombination reduces the absolute value of the LD. In the case of a negative LD, recombination thus increases the frequency of the extreme haplotypes at the expense of the intermediate ones (black arrows). Under negative LD, recombination thus increases the fitness variance in the population. **(b)** epistasis among loci. No epistasis (solid line) corresponds to multiplicativity of fitness effects and thus a linear relation between log fitness and number of mutations. Negative (dotted line) or positive (dashed line) epistasis between deleterious mutations implies that mutations interact synergistically or antagonistically in reducing fitness. (We assume for the sake of illustration that the fitness of haplotypes *aB* and *Ab* are identical). **(c)** regions where recombination is favored (green, selection against recombination; yellow, selection for recombination). Whenever $LD < 0$, recombination increases the efficiency of selection by increasing fitness variance (long-term advantage). When LD and epistasis are of opposite signs, recombination increases the mean fitness of the offspring compared to the parents (short-term advantage). In region 1, the long- and short-term advantage select for higher recombination. In region 2, the short- and long-term effects both select for lower recombination. In regions 3–6, selection for recombination depends on the relative magnitude of the short and long-term effects.

reproduce sexually, even though this mode of reproduction imposes a twofold cost (of producing males). Minimally, a theory for the maintenance of sex must explain why an asexual mutant cannot invade and eventually replace the sexual population. The particular challenge for such a theory is that it has to propose a benefit that is strong enough to counter the twofold cost. The MDH was originally formulated in this context [4,6]. To compensate a twofold cost the MDH requires, in addition to negative epistasis, a substantial load of deleterious mutations. More specifically, it requires that the rate of deleterious mutation per genome per replication cycle (U) is larger than one [4]. The magnitude of U in eukaryotes is controversial, with estimates ranging over several orders of magnitudes for different organisms. However, recent estimates, based on direct sequencing, suggest that at least for some eukaryotic species, U lies close to one [8,9]. A more detailed discussion of estimates of U can be found in Refs [10,11].

Second, what is the advantage of high compared to low recombination rates? Recombination breaks up combinations of co-adapted genes, thereby creating substantial fitness costs. Therefore, one has to explain why an individual with a lower rate of recombination cannot invade and replace the resident population, thus causing recombination rates to evolve successively to smaller values. Similar to the mechanism proposed by the MDH for the advantage of sexual over asexual reproduction, negative epistasis between deleterious mutations can also explain the maintenance of high recombination rates [3]. However, different conditions apply in the context of the evolution of recombination. First, there is no twofold cost of sex and, thus, there is no need for $U > 1$. Second, higher recombination rates are selected for only if epistasis is both negative and weak [12]. This is the case because typically the genes controlling recombination rates are only loosely linked to the loci under selection. The condition of weak epistasis does not appear in the context of the advantage of sexual over asexual reproduction, because there is no genetic exchange between sexual and asexual individuals.

Any explanation for the evolution of a reproductive strategy that involves genetic shuffling requires an answer to both these questions. Thus, negative epistasis is a necessary (but not sufficient) condition to explain the evolution of genetic shuffling by the MDH.

A suitable theory?

In addition to epistasis-based theories, there are other hypotheses that could account for the evolution of recombination [7], although, similar to the epistasis-based theories, they can explain an advantage of recombination only under specific (and often restrictive) conditions. Because it is likely that the correct explanation will emerge from one or a combination of the existing hypotheses, we think that to make progress in this field it is necessary to concentrate on assessing the plausibility of existing theories rather than developing new ones. Our goal here is to assess the plausibility of the MDH (and related hypotheses that are based on negative epistasis between deleterious mutations) in the light of recent experimental and theoretical studies. To this end, we review the experimental data [13–28], which, in our view, provide no support for the MDH, because there is currently no convincing evidence for a predominance of negative epistasis across many taxa. On the contrary, recent data suggest that, at least in viruses, positive epistasis predominates. However, the interpretation of such experimental data is fraught with difficulties. Therefore, we also discuss how far these difficulties weaken the experimental evidence that appears to contradict the MDH.

After reviewing the empirical data, we turn to theory. Recent theoretical challenges to the MDH come mainly from population genetic models that include both drift and epistasis. These models suggest that, for realistic parameters, the effect of epistasis on the evolution of recombination is negligible compared with the effect of drift. In conclusion, we argue that, although a role of negative epistasis in the evolution of recombination cannot be excluded, the MDH is losing ground as a theory for the evolution of recombination.

Experimental data on epistasis for fitness

Largely because of the connection between epistasis and the evolution of recombination, considerable effort has been put into measuring whether positive or negative epistasis predominates. As the evolution of recombination depends strictly on epistasis for fitness (and not on epistasis for other phenotypic traits), we focus here on recent microbial studies, as laboratory estimates of fitness-related traits in these organisms (i.e. growth rates) provide reasonably accurate proxies for fitness in the natural environment.

A variety of methods has been used to measure epistasis in microbial organisms, from viruses to eukaryotic microbes (Box 2). Until recently, the results of such studies were largely inconsistent: interactions exhibiting both positive and negative epistasis were measured, but the typical result of earlier studies was no significant predominance of either form (Box 2, Table I). However, most recent studies, usually with increased statistical power, have shown significant positive epistasis (although with a large variance).

Evidence from eukaryotic microbes

In eukaryotic microbes, epistasis has been measured for the fungi *Aspergillus niger* and *Saccharomyces cerevisiae* as well as for the green alga *Chlamydomonas moewusii*. Two studies have been conducted in *C. moewusii*; in the first, epistasis was found to be significantly negative for two fitness-related traits, growth rate (r) and carrying capacity (K) [18]. In the second study, epistasis was negative for K and absent for r [16]. In *A. niger*, epistasis for growth rate was positive, although not significantly different from zero [17]. The most recent eukaryotic microbial studies have all been conducted in *S. cerevisiae*; two of these found no evidence for a predominance of either positive or negative epistasis [23,25], whereas a third study [26], conducted on a much larger scale, found significant positive epistasis.

Evidence from bacteria

For bacteria, epistasis for competitive fitness was first tested in *Escherichia coli* and was found to be weakly

Box 2. Experimental approaches to measuring epistasis

Several methods exist for determining the type of epistatic interactions that operate between loci. These methods can be roughly divided into three categories: (i) those that look for epistasis by measuring interactions between pairs of loci; (ii) those that examine larger numbers of deleterious mutations (or proxies of mutations) to look for departure from a log-linear fitness decline; and (iii) those that compare the fitness distributions of parents and offspring (Table I).

Table I. Studies measuring epistasis in microbial organisms and viruses

Group	Organism	Method[b]	Result[a]	Refs
Eukaryotic	*Chlamydomonas*	3	Negative	[18]
	moewusii	3	Neg./Absent	[16]
	Aspergillus niger	1,2	Positive (n.s.)	[17]
	Saccharomyces	3	Absent	[25]
	cerevisiae	1	Absent	[23]
		1	Positive	[26]
Prokaryotic	*Escherichia coli*	1, 2	Positive (n.s.)	[20]
	Salmonella enterica	2	Positive	[21]
Viral	FMDV	2	Negative (n.s.)	[19]
	Polio virus	2	Positive (n.s.)	[15]
	Bacteriophage Φ6	2	Positive	[14]
	VSV	1	Positive	[22]
	HIV-1	1, 2	Positive	[13]
		2	Absent	[24]
		1	Positive	[27]
	Bacteriophage ΦX174	2	Positive	[28]

[a]n.s.: not significant.
[b]Method 1, direct measures of two-locus epistasis; Method 2, measuring the curvature of the fitness function; Method 3, comparing parent fitness with offspring fitness.

Direct measures of two-locus epistasis

Method 1 requires the ability to generate single mutations within lineages that can then be combined by crossing lineages [16,20,22,23,26] or similar methods [27]. The fitness values of the wildtype (w_{AB}), the two single mutants (w_{Ab}, w_{aB}), and the double mutant (w_{ab}) are then measured, and the epistasis is calculated as $\varepsilon = w_{AB}w_{ab} - w_{Ab}w_{aB}$. This method has two main advantages: the distribution of epistatic effects can be examined, and synthetic lethal mutations are included. The disadvantage is that, if epistasis is weak, it can be difficult to gain enough statistical power. One alternative way to acquire pairwise measures of epistasis was used by Bonhoeffer *et al.* [13], who obtained estimates for the fitness values of each genotype (w_{AB}, w_{Ab}, w_{aB} and w_{ab}) by averaging over all backgrounds in which that allelic combination appeared, although these genotypes might have differed at a large number of other loci.

Measuring the curvature of the fitness function

In Method 2, in which the relationship between the number of deleterious mutations and fitness is analyzed, various techniques have been used to obtain strains with different numbers of deleterious mutations. These include mutation accumulation [13,19,20,28], chemical mutagenesis [15], site-directed mutagenesis [24], or crossing strains to obtain individuals containing different combinations of mutations [17]. The log-fitness values of each of these strains are then plotted against the number of mutations. Positive or negative curvatures of this fit are interpreted as a predominance of positive or negative epistasis (Figure I; Box 3). The disadvantage is that only average epistasis is quantified, although variance in epistasis can severely restrict the parameters under which recombination might be favored (see main text).

Comparing parent fitness with offspring fitness

In Method 3, parents with largely different numbers of mutations are mated. If the mean fitness of the offspring is larger or smaller than the mean fitness of parents, then this implies positive or negative epistasis, respectively (Figure I). There are several similar approaches in which parents with approximately equal numbers of mutations are crossed or which consider changes in the skew of the fitness distribution [16,18]. However, these approaches have been criticized for lacking accuracy [40].

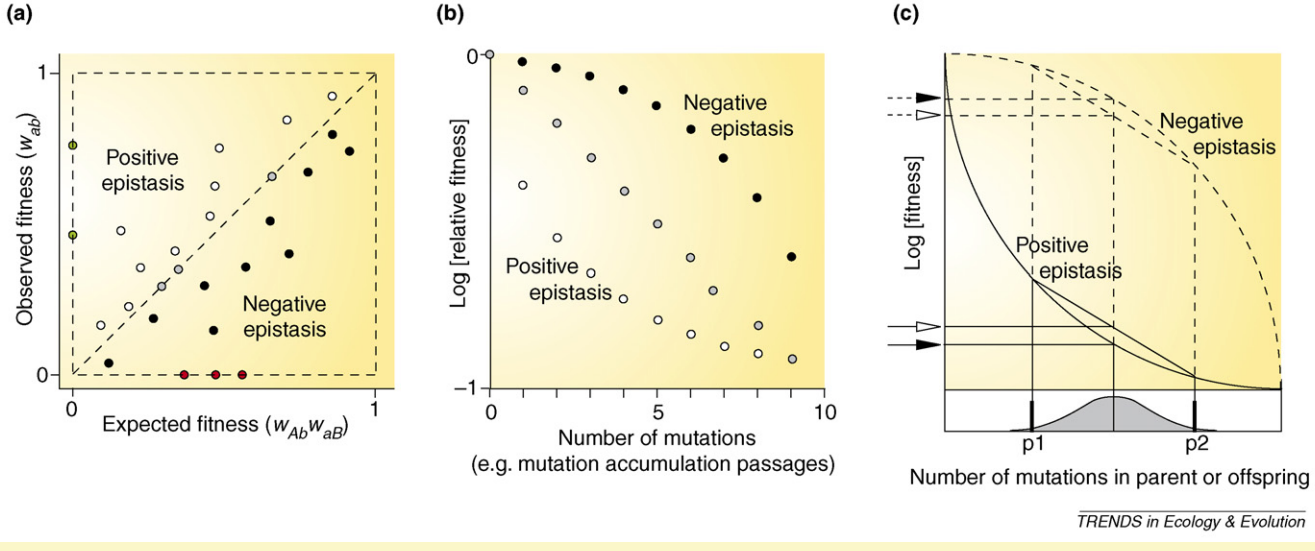

Figure I. Experimental approaches to measuring epistasis. **(a)** Method 1: fitnesses are expressed relative to that of the wild type (*AB*). The expected fitness of the double mutant (*ab*) is then calculated by multiplying the fitness values of the two single mutants (*Ab*·*aB*). Positive or negative epistasis implies that the fitness of the double mutant is higher or lower than expected (white or black circles); grey circles correspond to the absence of epistasis. Extreme forms of epistasis are illustrated by green circles ('compensatory viability') and red circles ('synthetic lethality'). **(b)** Method 2: if the fitness decline is accelerating, decelerating or constant, then negative, positive or no epistasis is inferred (black, white or gray circles); **(c)** Method 3: consider two parents, p1 and p2, with strongly different numbers of deleterious mutations (black bars); they have, by definition, a positive LD (i.e. the intermediate genotypes are underrepresented). Hence, if they are crossed, the number of intermediate genotypes increases (grey distribution). For positive or negative epistasis (full or dashed lines), these intermediate genotypes have a lower or higher fitness (filled arrowheads) than expected (open arrowheads) from the parental genotypes. Thus, on average, the offspring have a lower fitness than do the parents for positive epistasis; and the converse for negative epistasis.

positive, although not significantly different from zero [20]. This study was the first to show that many pairs of interactions exhibited either significant positive or negative epistasis, although the two forms were roughly equally common. A more recent study in *Salmonella* found evidence of significant positive epistasis [21]; interestingly, this appeared to rely on a physiological mechanism in which cellular chaperones that assist in protein folding were upregulated in cells with many deleterious mutations, hence reducing the impact of further deleterious mutations.

Evidence from viruses

Epistasis has also been assessed in several viral systems. The first experimental assessment, in foot-and-mouth disease virus (FMDV), found no significant evidence of epistasis [19]. An experiment in polio virus [15], although not directed specifically at testing for epistasis, found a non-significant trend of positive epistasis. More recent experiments have found significant evidence of positive epistasis acting between loci in HIV-1 [13,27], the segmented RNA bacteriophage ϕ6 [14], the DNA bacteriophage ϕX174 [28], and a segmented RNA virus, vesicular stomatitis virus [22]. Although both Bonhoeffer *et al.* [13] and Sanjuan *et al.* [22] found a large range of both positive and negative epistatic interactions among different pairs of mutations, mean epistasis was significantly positive for both studies. In addition, Burch and Chao [14], Bonhoeffer *et al.* [13] and Silander *et al.* [28] all found positive curvatures of the fitness functions (Box 2; Method 2). Finally, a recent study examined epistasis within the transcriptional promoter of HIV-1 and found no evidence of epistasis [24].

Caveats for the experimental data

The assessment of experimental studies requires some qualifications. In some cases, the absence of significant evidence for epistasis might be due to low levels of variation in fitness [23]; those studies that have found evidence for epistasis tend to have greater variation in fitness [13,14,22,26–28]. Second, few studies include an assessment of the frequency of synthetic lethality (i.e. the frequency of mutations that are lethal only when occurring in combination). This is the strongest possible type of negative epistasis and could, thus, have an important role in the evolution of recombination. (Compensatory viability presents an analogous form of strong positive epistasis, in which one or both of the single mutants are lethal, but the combination is viable; e.g. Ref. [27]). In many studies, the method used to measure epistasis is based only on fitness measures in viable genotypes and, thus, does not enable any assessment of synthetic lethality (or compensatory viability) [13,14,19].

Recently, Sanjuan and Elena [26] collected data from several previously published experiments and proposed that the dominant form of epistasis is correlated with genome complexity. Although this is an intriguing hypothesis, their conclusions should be taken with some caveats. Most studies that have reached statistical significance find that positive epistasis predominates. However, for methodological reasons, these studies mainly come from viruses, because these systems enable sufficiently large data sets to be generated. By contrast, most measurements in higher organisms have not shown significant deviations from zero epistasis [29]. Therefore, an apparent decrease in epistasis with increasing genomic complexity might be due to methodological reasons. Interestingly, the most recent and largest scale eukaryotic study did find significant evidence for positive epistasis [26]. In summary, we believe that it is early days to decide whether Sanjuan and Elena's hypothesis is borne out by the data, because their extrapolation for epistasis in higher organisms is based only on five data sets, and did not include a more recent study on yeast [27].

Difficulties in interpreting epistasis data

One difficulty in interpreting the data is that all studies that have measured epistasis between pairs of loci have found evidence for both positive and negative interactions. Using the mean value of epistasis to infer how selection acts on recombination tacitly assumes that positive and negative interactions of equal strength cancel out. However, several studies show that this is incorrect.

First, Otto and Feldman [30] have argued that variance in epistasis selects for smaller recombination rates. More specifically, they showed that, in the absence of correlations between epistasis and the strength of selection on the interacting loci, selection for an allele that encodes higher recombination decreases as variation in epistasis increases. Because most studies have detected considerable variation in epistasis, Otto and Feldman's study further decreases the support for the MDH. However, this result was derived under the assumption that the modifier of recombination is weakly linked to the rest of the genome. Therefore, the result applies to the problem of high versus low recombination rate rather than that of sexual versus asexual reproduction.

Second, Kouyos *et al.* [31] showed that a symmetrical distribution of epistatic effects with a mean of zero does not generally generate a symmetrical distribution of LD with a mean of zero. In fact, it can generate a distribution of LD with either a positive or negative mean, depending on the correlation between epistasis and the strength of selection. Moreover they showed that the distribution of epistatic effects alone does not determine whether recombination will be selected for or against. Further analysis showed that epistatic interactions of a given strength can create LD (and selection for or against recombination) ranging over several orders of magnitude and that the level of LD created depends crucially on the strength of selection at the interacting loci: the interactions that strongly affect the evolution of recombination are those with weak selection against single mutants and strong selection against the double mutants and those interactions in which there is strong selection against the single mutants but weak selection against the double mutants. Conversely, sets of mutations with strong selection against all combinations contribute little to the LD because selection results in negligible polymorphism at the involved loci.

Inferences on the selection for recombination that are based on the curvature of the decline of log fitness with the number of mutations (Box 2) suffer from the same problem. The disproportionate effect of weakly selected interactions

Box 3. The predictive power of the fitness function

A common way to estimate the form of epistasis is via the fitness function curvature. The fitness function is usually obtained by regressing log-fitness against the number of mutations (n) using a quadratic function of the form $\log(w) = a*n + b*n^2$, where w is the fitness, n is the number of mutations, and a and b are fitting parameters. A positive curvature ($b > 0$) is interpreted as positive epistasis and a negative curvature as negative epistasis.

Here, by means of a simple simulation we show that a linear decline of log fitness does not imply a vanishing LD. To this end, we consider sequences consisting of nine biallelic loci. The fitness landscape is constructed by superimposing a random term to a multiplicatively declining fitness function (Figure Ia): The wild type has fitness 1. The log fitness of a sequence i (other than the wild type) is the sum of a first term that declines linearly with the Hamming distance (which counts the number of loci with different alleles) between i and the wild-type [slope $a = log(0.9)$] and a second random term chosen uniformly between $-a/2$ and $a/2$. By definition, this fitness landscape corresponds to a fitness function with no curvature (Figure Ib). Hence, if the curvature of the fitness function were a good predictor of LD, such fitness landscapes should generate no LD on average. In Figure Ic, we show the average LD at mutation selection balance for 1000 random realizations of such fitness landscapes. The corresponding histograms show that negative linkage disequilibria predominate, despite the fact that the fitness functions have no systematic curvature.

The reason for this discrepancy is as follows: Although the random error generates equal amounts of positive and negative epistasis, in this specific fitness landscape, mutations with small selection coefficients tend to interact with negative epistasis, whereas those with large selection coefficients tend to interact with positive epistasis. Therefore, the polymorphism at mutation–selection balance is larger for the interactions with negative epistasis. A larger polymorphism, in turn, translates into larger LD. Thus, equal amounts of positive and negative epistasis translate into unequal amounts of positive and negative LD because interactions with negative epistasis are found more often in the population (because of their greater polymorphism). For the interpretation of fitness functions, this finding implies that the MDH can be consistent with the data, even when the average fitness decline exhibits little (or even positive) curvature. However, whether this is the case depends on: (i) the correlation between epistasis and the strength of selection; and (ii) how the LD distribution translates into selection for or against recombination. Regarding the first condition, it is interesting that a negative correlation between epistasis and selection strength has been found *in silico* [41–44].

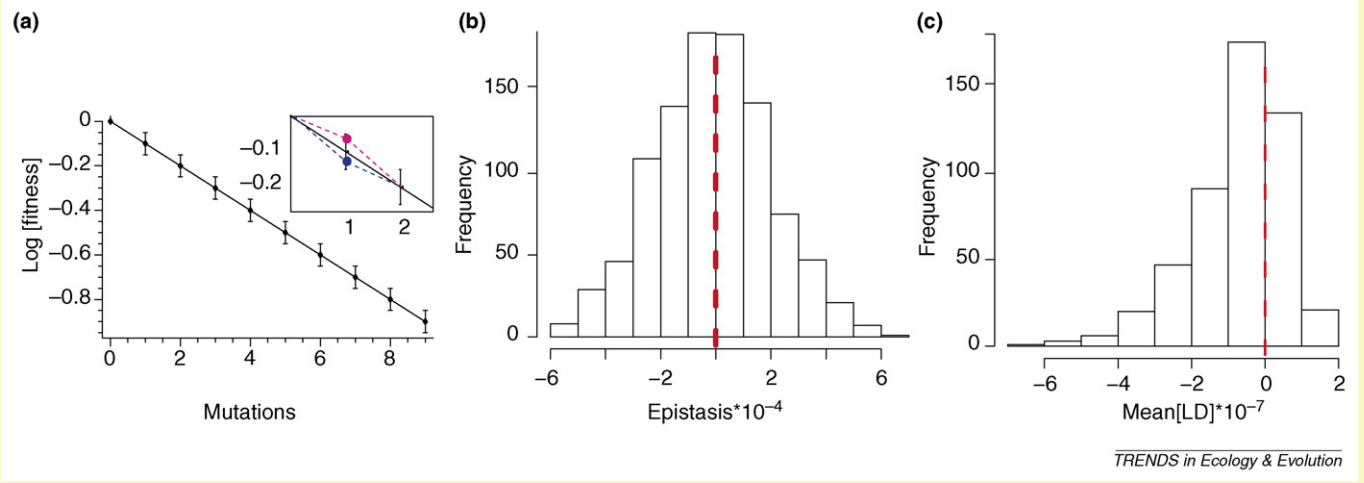

Figure I. Predictive power of a linear fitness function. **(a)** illustrates the type of fitness landscape used in the simulations: the fitness of a genotype with n mutations is drawn randomly from the range indicated by the corresponding bar. The inset illustrates that, in this particular type of fitness landscape, interactions with negative epistasis (red) tend to be under weaker selection than are those with positive epistasis (blue). **(b)** shows the distribution of epistasis, as measured by the curvature of the fitness function ($-b$), for 1000 realizations of the fitness landscape. **(c)** shows the resulting distribution of the average LD (mutation rate used $m = 10^{-4}$). Although the epistasis distribution is centered on zero, the average-LD distribution has a negative mean. The dashed lines in (b) and (c) indicate zero epistasis and LD, respectively.

on LD makes the curvature of the fitness decline a poor predictor of the LD distribution (Box 3).

The above considerations illustrate that the strength and sign of epistasis does not fully determine the selection for recombination. More generally, the relevance of a given epistatic interaction for the evolution of recombination is not only determined by the strength of this interaction, but also by the frequency with which this interaction is found in the population. Most experimental studies measure physiological epistasis [32]; that is, the physiological effects of an epistatic interaction, independent of its frequency in the population. By contrast, theoretical studies (which measure the selection for or against recombination) are based on population epistasis [32]: the effect of an epistatic interaction, weighted by its frequency in the population. Clearly, measuring population epistasis in natural populations will be difficult. However, it is population epistasis that ultimately matters for the evolution of recombination, because the selection for recombination depends on the strength of the epistatic interactions and on the frequency with which such allelic combinations occur in natural populations.

Theoretical challenges against the role of epistasis

In addition to negative epistasis, drift in concert with selection can generate negative LD. This occurs in the following way (known as the Hill–Robertson effect [33]): first drift (i.e. random sampling) causes genotype frequencies to deviate randomly from their expected values, thus creating deviations from the LD that is generated by selection. These deviations are unbiased (i.e. drift generates as much positive as negative deviation from the level of LD expected from selection). However, selection acts differently on positive and negative LD: positive LD increases the efficiency of selection (because the extreme genotypes are overrepresented and, therefore, variance in fitness is increased), whereas negative LD decreases the

efficiency of selection (because the extreme genotypes are underrepresented and, therefore, variance in fitness is decreased). As the efficiency of selection increases with increasing fitness variance, selection reduces positive LD faster than it does negative LD. In summary, drift together with selection generates a tendency towards negative linkage disequilibria.

The Hill–Robertson effect is important because it competes with epistasis in creating LD; indeed, it can override the effects of epistasis [34]. The concept behind the effect is not new; however, the notion that it is a stronger force than epistasis in generating negative LD has recently received support from several theoretical studies.

Otto and Barton [34] and Keightley and Otto [35] compared the relative importance of the Hill–Robertson effect and epistasis in generating LD under different selection regimes. In particular, they studied selection on a locus that increases recombination (a recombination modifier). This modifier is linked to a genome that is either subject to beneficial mutations [34] (directional selection) or to deleterious mutations [35] (purifying selection). In both cases: (i) higher recombination rates are selected for even in the absence of epistasis; and (ii) including additional epistatic interactions (either positive or negative) in the fitness landscape has weak effects on selection for the recombination modifier (compared with the Hill-Robertson effect). With only two fitness-affecting loci, the Hill–Robertson effect causes substantial selection for high recombination rates only when the population size is not too large (i.e. $<10^4$–10^5 [34]). However, this does not invalidate the importance of the effect in general, because Iles *et al.* [36] and Keightley and Otto [35] have shown that, for multiple loci, higher recombination rates are selected for even in larger populations. In addition, Martin *et al.* [37] showed that population structure can further broaden the range of population sizes for which the Hill–Robertson effect selects for higher recombination rates. In summary, these studies suggest that, compared with the Hill-Robertson effect, epistasis has only a marginal role in theories explaining the evolution of recombination through an increased response to selection.

Although these studies convincingly demonstrate the power of the Hill-Robertson effect, it will require more work to establish whether negative LD created through the interplay of drift and selection works as a universal explanation for the maintenance of sexual reproduction and recombination. First, for most parameters values explored in these studies, the strength of the Hill–Robertson effect (i.e. the selection on a recombination modifier) is small. Thus, it is implausible that the effect compensates fully for the large disadvantage of sexual reproduction (in terms of the cost of producing males), and additional mechanisms (which might involve epistasis) are required to explain the maintenance of sexual reproduction. Second, although these studies have implied that negative epistasis has only a peripheral role in the evolution of recombination, the simulations do show a strong effect of epistasis for some parameters values. In particular, the study of Keightley and Otto [35] shows that strong negative epistasis can increase the selection on a recombination modifier up to a factor of ten (for an intermediate genomic mutation rate $U = 0.1$).

Box 4. Outstanding questions

- Are physiological and population epistasis qualitatively different? As recombination works on the basis of the genetic variation that exists in natural populations, it will be necessary to measure epistatic interactions as they occur in these populations.
- Does positive epistasis also predominate in higher organisms? Most significant results have been based on studies of viruses, because of the ease with which one can generate mutants and measure their fitness in sufficient numbers. It will be necessary to address these questions with high throughput techniques in higher organisms to determine what type of epistasis predominates in such organisms.
- Are epistatic interactions of sufficient strength and do they fluctuate fast enough to support the Red Queen Hypothesis? Although the current evidence does not support a significant role of epistasis in the evolution of recombination under the mechanism suggested by the MDH, it is conceivable that other epistasis-based theories, such as the Red Queen Hypothesis, provide the explanation. However, these theories also make strong assumptions about the nature of epistatic interactions, which need to be empirically tested.

Is epistasis relevant for the evolution of recombination?

In our view, recent theoretical and experimental evidence presents a considerable challenge for theories of the evolution of recombination based on negative epistasis. The main challenge emerging from theoretical studies is that the role of epistasis in generating negative LD might generally be weaker than that of drift. This was shown both for beneficial [34] and detrimental mutations [35]. On the experimental side, there are an increasing number of studies reporting a predominance of positive epistasis, first in viruses and, more recently, for both prokaryotic and eukaryotic species. Clearly, there is no general predominance of negative epistasis as would be required to support the MDH (and related theories). However, as we point out, there are difficulties in the interpretation of epistasis data. In essence, these arise because weakly selected interactions are expected to have strong effects on LD and, thus, on the evolution of recombination. We emphasize, however, that these difficulties do not support the MDH, but only weaken the evidence against it.

Neither the theoretical nor the experimental evidence provides a watertight argument to reject the MDH and many questions remain (Box 4). However, with increasing evidence against it, and little evidence in support, the MDH is losing ground as a favorite theory for the evolution of recombination.

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