The evolution of sex: empirical insights into the roles of epistasis and drift

J. Arjan G. M. de Visser and Santiago F. Elena‡*

Abstract | Despite many years of theoretical and experimental work, the explanation for why sex is so common as a reproductive strategy continues to resist understanding. Recent empirical work has addressed key questions in this field, especially regarding rates of mutation accumulation in sexual and asexual organisms, and the roles of negative epistasis and drift as sources of adaptive constraint in asexually reproducing organisms. At the same time, new ideas about the evolution of sexual recombination are being tested, including intriguing suggestions of an important interplay between sex and genetic architecture, which indicate that sex and recombination could have affected their own evolution.

Recombinational load
A decrease in mean fitness due to the break up of co-adapted (that is, epistatic) gene combinations by recombination.

Drift
A change in genotype frequencies due to chance variation. Also known as random or genetic drift.

Epistasis
Deviation from independent (that is, multiplicative) gene action on a polygenically encoded phenotype, or on fitness.

**Laboratory of Genetics, Wageningen University, 6703 BD Wageningen, The Netherlands.*
‡Instituto de Biología Molecular y Celular de Plantas, Consejo Superior de Investigaciones Científicas-UPV, 46022 València, Spain.
Correspondence to J.A.G.M.d.V. or S.F.E.
e-mails: arjan.devisser@wur.nl; sfelena@ibmcp.upv.es
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Sexual reproduction, in one form or another, is present in all branches of the tree of life. The fact that sex is so phylogenetically widespread indicates that it comes with a simple and general advantage that explains its evolutionary success. In fact, sexual reproduction often comes with substantial costs[1–4]. First, for species in which males contribute no other resources than genes to the offspring, a mutation that has the sole effect of causing females to reproduce asexually will inevitably succeed because its frequency doubles at each generation (the classical 'twofold cost of sex'). Second, offspring that are produced by sexual reproduction could suffer fitness losses from the breaking up of co-adapted gene combinations (recombinational load), which remain linked together in asexual offspring. Third, in species with separate sexes, fecundity depends on an ability to bring gametes into contact with those from the opposite sex, which might be problematic when densities are low; by contrast, asexual reproduction does not suffer from such uncertainties. There are also several other costs that apply to more specific cases, such as the time, energy and risks (of predation or infection with parasites and selfish genetic elements) that are involved in the act of sex.

A 'simple' explanation for the benefit of sex has remained elusive for more than a century. Nevertheless, in recent years the search has taken several new avenues, stimulated by frustration with the lack of evidence for formerly popular ideas and the presentation of new opportunities associated with recent genomic technologies, the development of novel experimental and bioinformatics tools and new theoretical models.

Here we first discuss the main theoretical models for how sexual recombination might have evolved, focusing on those in which sex has an indirect benefit that allows the organism to better respond to selection. Recent empirical evidence relating to these models is discussed, distinguishing between the evidence for each of the two evolutionary mechanisms that have been proposed to be responsible for adaptive constraints in asexuals — drift and negative epistasis (ε). We then examine new models for the evolution of sexual recombination that have been proposed in the absence of firm support for the longer-standing theories. Last, we discuss new evidence for a two-way relationship between sexual recombination and genetic architecture, which provides insights into how sex might promote its own maintenance.

Direct and indirect models

Numerous theoretical advantages of sex and recombination have been postulated. These ideas can be roughly divided into two categories: those in which there is an immediate direct benefit of sex due to the increased mean fitness of offspring in the next generation, and those in which the advantage is indirect and relies on the increased genetic variation for fitness that is produced after sex, which can be used by natural selection — the so-called 'variation-and-selection models'[5].

Direct models. Two hypotheses that involve immediate advantages have been explored in detail. The first is that sex and recombination are by-products of the evolution of dsDNA repair — for which a diploid phase is necessary[6]. The second is that they are the by-products

Box 1 | Experimental evidence for direct benefits of sex

Explanations for the evolution of sex that propose a direct fitness benefit have received little attention, probably because they are unable to explain the evolutionary maintenance of sex and recombination. One recent study nevertheless found indirect support for an association between sex and DNA repair by examining factors that induce sex in the multicellular green alga *Volvox carteri*, which is facultatively sexual[113]. By using two different antioxidants to manipulate intracellular levels of reactive oxygen species (ROS), it was shown that overproduction of ROS is part of the proximate mechanism that induces sexual reproduction in this species. Because stress conditions typically induce sex in many facultatively sexual species, such as most unicellular eukaryotes, and because several stresses are thought to induce ROS levels, the connection between sex and ROS might hint at the ultimate reasons for the origin of sex.

Another direct benefit of sex was recently shown in a mutation-accumulation study that used the facultatively sexual fungus *Aspergillus nidulans*[114]. Lineages that were subjected to conditions that resulted in mutation accumulation and were maintained by transferring single sexual spores showed slower fitness declines than equivalent lineages in which single asexual spores were used. This was surprising, because the sexual spores were produced by selfing in this homothallic species, so recombination had little effect on genetic variation, which is considered to be the main route by which sex can lead to increases in fitness (see main text for details). Therefore, the slower fitness decline in the sexual lineages was interpreted as resulting from more stringent selection against early progeny carrying deleterious mutations, dubbed as a 'selection arena'[37,38]. Although it remains unclear why intra-organismal selection, like other secondary advantages such as a thicker spore wall, would evolve particularly in the sexual route, the apparent association between sex and secondary adaptations clearly contribute to the evolutionary maintenance of sex and recombination.

Anisogamy
The condition in which the male and female gametes are of different sizes.

Sexual selection
Selection among individuals of one sex that is exerted through competition for mates, or through the mating preference of the opposite sex.

Directional selection
Natural selection that promotes the continued change of a phenotype in one direction.

Linkage disequilibrium
Deviation in a population from a random distribution of alleles at different loci, denoted by D. $D < 0$ reflects the relative absence of individuals with particular allele combinations, $D > 0$ reflects the relative abundance of such individuals.

Stabilizing selection
A form of selection that results in intermediate phenotypes having greater fitness than extreme phenotypes.

Fisher–Muller hypothesis
The theory that, through recombination, sex speeds up adaptation by bringing together beneficial mutations that have arisen in different genetic backgrounds.

of selfish genetic elements that enhance their own spread during sex[7–9]. Both mechanisms could have been involved in the origin of sex, but cannot explain its maintenance, because sex is not necessary for dsDNA repair, for which a diploid phase suffices, and selfish elements can no longer enhance their own spread by inducing sex once they reach a high frequency. However, the distinction between direct factors that might have been involved in the evolutionary origin of sex and recombination versus those that might have been involved in its maintenance is complicated by adaptations associated with sexual reproduction that arose after its origin, such as anisogamy, sexual selection and the stress-resistance of sexual propagules[10]. Although the association of these secondary adaptations with sexual reproduction might reflect more fundamental evolutionary advantages of sex, this is difficult to study without the ability to separate the different adaptations in experimental or comparative studies. For this reason, we concentrate on factors that are relevant for the maintenance, rather than the origin, of sex and recombination. A brief overview of recent empirical evidence for the direct benefits of sex is provided in BOX 1.

Indirect models. The most widely studied hypotheses for the benefits of sex are the variation-and-selection models (reviewed in REF. 5). These models assume that sex is maintained because of an indirect benefit whereby sexual recombination facilitates the population's response to directional selection. Such a benefit is achieved by increasing the useful genetic variation through the reassortment of alleles. This can either speed up adaptation or slow down maladaptation in the face of recurrent deleterious mutations. Although this postulated benefit of sex is indirect, it can still be substantial and work at a relatively short timescale if selection is sufficiently strong[11–13].

Requirements for variation and selection models

The crucial prerequisite for sex to facilitate selection is that the genetic variation for fitness in the population is lower than for a random distribution of alleles. In other words, linkage disequilibrium (D) should be negative, with certain allele combinations being present in the population at a lower frequency than expected by chance. Negative linkage disequilibrium can result from directional selection following the effects of drift. Another possible source of negative linkage disequilibrium is negative epistasis ($\varepsilon < 0$), the situation in which alleles with negative effects on a phenotype or fitness interact synergistically (enhancing each other's detrimental effects), or in which alleles with positive effect interact antagonistically (diminishing each other's advantageous effects). The effects of qualitatively different forms of epistasis on the influence of recombination on mean and variance of fitness in the next generation are illustrated in FIG. 1. If selection is not directional but stabilizing, sex might also increase genetic variation, but this variation is selected against[14,15]. However, we should emphasize that drift and negative epistasis are not mutually exclusive mechanisms, and might well operate together. Indeed, drift will always affect the genetic make up of populations because all populations have finite size, but its importance relative to negative epistasis remains an open question.

The variation-and-selection explanations of sex can be classified by the postulated source of negative linkage disequilibrium — that is, drift or negative epistasis. For several of these models, the source is directional selection combined with drift in finite populations. One of these, the Fisher–Muller hypothesis, proposes that in finite populations the beneficial mutations that are responsible for adaptation arise in linkage disequilibrium due to their low rate of occurrence[11,13,16–20]. If, by chance, they arise with $D > 0$, sex is not beneficial, but selection tends to restore equilibrium by benefiting individuals with multiple beneficial mutations. By contrast, if the mutations arise with $D < 0$, selection will strengthen this disequilibrium because the best genotype does not yet exist or is not found at high frequency[4]. Therefore, on average, selection plus chance create negative linkage disequilibrium, leading to an advantage for sexual recombination.

Another theory that relies on drift is the Muller's ratchet hypothesis[21,22], which assumes that chance processes in small populations are responsible for the gradual and irreversible accumulation of deleterious mutations. The loss of individuals with few mutations causes a distribution of deleterious alleles in which $D < 0$, and allows recombination to restore a random distribution by recreating mutation-free individuals. Last, the background selection hypothesis[23–25] states that sex helps to liberate beneficial mutations from genomes that carry deleterious alleles. This hypothesis also invokes drift as the ultimate source of $D < 0$. In this case, $D < 0$ results from associations between beneficial and deleterious alleles[24–26].

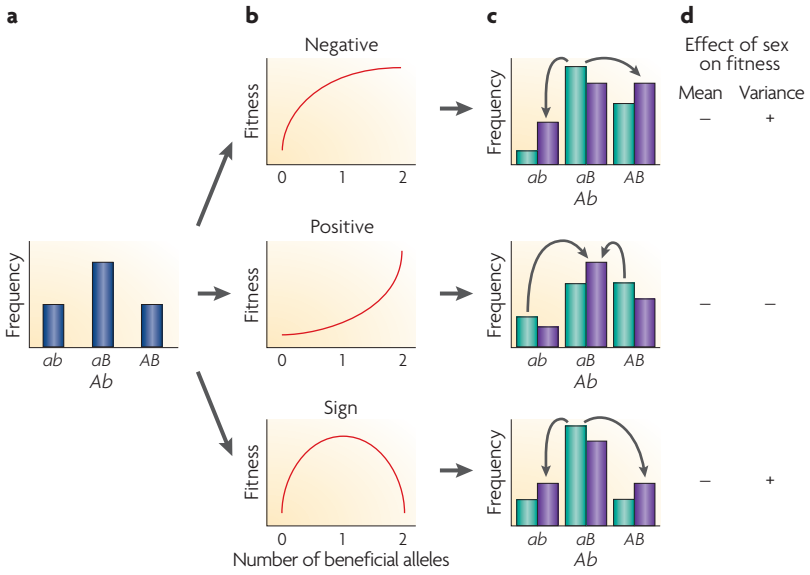

Figure 1 | **Epistasis, linkage disequilibrium and the direct and indirect effects of sex.** Qualitatively different forms of epistasis can exist among beneficial alleles; in some situations, this can provide the conditions for an indirect advantage of sex in a population in which two loci are occupied by either low-fitness alleles (*a* and *b*) or high-fitness alleles (*A* and *B*). **a** | A population in linkage equilibrium ($D = 0$), for which sex has no effect on fitness. **b** | Three forms of epistasis between the beneficial alleles: negative epistasis ($\varepsilon < 0$), in which the beneficial alleles diminish each other's benefit when combined (top panel); positive epistasis ($\varepsilon > 0$), in which the beneficial effects of the same two alleles are amplified when the two are combined (middle panel); and sign epistasis, in which the two single mutants, *aB* and *Ab*, have higher fitness than both extreme genotypes, *ab* and *AB*, (bottom panel). **c** | The particular form of epistatic selection that occurs will affect the distribution of genotypes in the next generation (shown as green bars). Recombination will subsequently affect genotypic frequencies (shown as purple bars); the net production of new genotypes is indicated by arrows. Both negative and sign epistasis lead to $D < 0$ and therefore decreased variance, whereas $\varepsilon > 0$ causes $D > 0$ and increased variance. **d** | The direct effect (on mean fitness) and indirect effect of sex (on fitness variance). All three forms of epistasis cause an immediate fitness decline as a result of sex (that is, recombinational load), due to the production of low-fitness offspring. The indirect effect on fitness variation is negative under $\varepsilon > 0$. Under sign epistasis, recombination does increase the genetic variation, but this variation is deleterious as it generates more offspring with fitness levels that are further from the optimum. Besides drift, only $\varepsilon < 0$ (top panel) can cause a faster response to selection in the long term. A more comprehensive theoretical discussion of the short- and long-term effects of sex and recombination as function of D and ε is given in REF. 4.

Muller's ratchet
The process by which the genome of an asexual population accumulates deleterious mutations in an irreversible manner, owing to the chance loss of individuals with the lowest number of mutations.

Background selection
The interfering effect of selection against deleterious mutations at loci that are linked to another locus that is under positive selection.

In large populations, for which chance is less important, selection can cause $D < 0$ if selected alleles show negative epistasis. Hypotheses for the evolution of sex that belong to this category include the mutational deterministic hypothesis (MDH)[27,28], which postulates a more efficient removal of deleterious mutations when $\varepsilon < 0$, provided that epistasis is weak enough to limit the negative direct effects of sex on mean fitness (FIG. 1). The shifting optimum models[1,15,29–31] also rely on negative epistasis. In these models, sex enhances the response to selection in environments in which changing abiotic or biotic factors determine the optimal phenotype, although the beneficial effect of sex under such conditions can vanish if selection is weak[32].

Below, we examine the empirical evidence for key aspects of the variation-and-selection models. We start with the question of whether sex can aid selection by either reducing mutational load or increasing the rate of adaptation. We then examine evidence for the two proposed sources of the negative linkage disequilibrium that is necessary for sex to evolve: drift and negative epistasis.

Tests for the effect of sex on selection

Reducing the deleterious mutation load. It was shown more than a decade ago that sex reverses the effects of Muller's ratchet in the segmented bacteriophage ϕ6 (REFS 33,34). Two recent studies have used comparative methods to further address this question by taking advantage of the rapidly growing wealth of genomic data. Deleterious mutations in mitochondrial protein-coding genes were found to have accumulated at a fourfold-higher frequency in obligate asexual lineages than in sexual lineages of the microcrustacean *Daphnia pulex*[35]. Mitochondrial genomes provide two advantages for such studies[35]: they have elevated mutation rates, which enhances the power for detecting excess rates of mutation accumulation is short-lived lineages, and mitochondrial genomes are haploid, so sequencing is largely simplified. However, because mitochondria usually do not recombine, the inference of the effect of recombination on the accumulation of mutations in the mitochondrial genome was indirect in this case, and was proposed to rely on selective interference between the mitochondrial genes and the entire nuclear genome, with the nuclear genome being subject to recombination in the sexual lineages.

In a second study, in contrast to the findings from *Daphnia pulex*, no difference in the frequency of deleterious mutations between the selfing species *Caenorhabditis elegans* and *Caenorhabditis briggsae* and the outcrossing species *Caenorhabditis remanei* was observed[36]. In both studies, explanations for differences in mutation accumulation other than the presence or absence of sexual reproduction must also be considered, such as a more stringent 'selection arena'[37,38] during sexual reproduction, or differences in the effective sizes of sexual and asexual populations. Altogether, comparative genomics studies have provided mixed evidence for the importance of sex in purging deleterious mutations.

The effect of recombination on mutation clearance can be studied more directly in evolution experiments. In a recent study that used digital organisms[39] for this purpose[40], sexual populations survived Muller's ratchet better than asexual populations at very small population sizes, for which many instances of mutational meltdown[41] were observed. However, they also accumulated more deleterious mutations at slightly larger population sizes. Interestingly, when all possible random mutations were introduced in the surviving digital creatures, a lower fraction seemed to be lethal and a higher fraction seemed to be mildly deleterious in the sexual compared with the asexual organisms, which might explain the unanticipated higher fitness of the asexuals. A possible reason for the differences in fitness effects of mutations in sexual and asexual organisms involves differences in genome architecture, which we discuss below.

Segmented bacteriophage
A bacterial virus that has a genome that is divided into several fragments (chromosomes), which can be encapsidated into either the same or different viral particles, and allows reassortment of segments in co-infected bacteria.

Selective interference
The situation in which the fixation of an allele at a locus affects the probability of fixation of alleles at other linked loci.

Digital organisms
Computer-generated organisms that mutate and evolve inside the memory of a computer, where they compete for CPU time, on which they depend for their replication.

Mutational meltdown
The process by which small populations accumulate deleterious mutations leading to loss of fitness and a concomitant decline in effective population size. The decline in population size further accelerates Muller's ratchet and enhances the likelihood of extinction.

Although purging deleterious mutations by recombination might be one advantage of sex, other factors might subtract from this benefit. In viruses, the effects of sex and recombination become apparent only if two or more genetically different viruses co-infect a host cell. Froissart *et al.* used ϕ6, which has a genome that consists of three segments, to test the effect of sex on the removal of introduced deleterious mutations[42]. Because sex in this virus depends on co-infection and reassortment of parental segments in the offspring, it has two opposing effects: besides causing recombination, it also shields mutations from selection by complementing deleterious segments. Indeed, when segments containing a single harmful mutation were mixed with the wild-type virus, selective clearance of these mutations was slower in sexual populations than in asexual populations. So, at least in segmented viruses, sex might not be of great help in removing deleterious mutations.

Increasing the rate of adaptation. What is the empirical support for the faster rate of adaptation of sexual populations to novel or variable environments? The yeast *Saccharomyces cerevisiae* has been widely used to address this question, owing to the ease of manipulating and inducing sex. However, the results of such studies have been variable. It was shown that periodic sex did not speed up the rate of adaptation to a new environment, but was useful in removing deleterious mutations from populations that are replicating in a constant environment[43]. By contrast, other studies in this species[44] and in the unicellular alga *Chlamydomonas reinhardtii*[45] showed that when sexual and asexual populations were adapting to a new environment, sexuals tended to win.

Two recent studies have also tested the effect of recombination on the rate of adaptation in evolving microbial populations. When populations of *C. reinhardtii* that initially lacked genetic variation were allowed to adapt to a novel growth medium in sexual and asexual populations of varying size, sex increased the rate of adaptation at all population sizes, but particularly in large populations[46]. All adaptively useful variation had to be created *de novo* in these populations, and larger populations were able to do this more rapidly. Adaptation showed diminishing returns from increases in population size in the asexual populations, indicating that it was hampered by clonal interference between genotypes that carried alternative beneficial mutations. Sex was proposed to release populations from this constraint by allowing beneficial mutations to come together, thereby accelerating adaptation.

Interestingly, another recent study that used initially diverse populations of the bacteriophage ϕ6 also found evidence that sex releases populations from the adaptive constraints of clonal interference[47]. In contrast to the *C. reinhardtii* study, the sex advantage that was observed for ϕ6 decreased rather than increased with increasing population size. These results were nevertheless interpreted as consistent with sex removing the impeding effects of clonal interference, because the presence of genetic variation together with the high mutation rate of ϕ6 generated sufficient genetic variation to cause clonal interference, even in small populations.

Two other studies took advantage of recent insights into the genetics of recombination in *S. cerevisiae* to construct sexual and asexual genotypes that were isogenic except for two knockout mutations in the meiosis genes *SPO11* and *SPO13*. The asexual double mutant is fully fertile and produces diploid spores that are identical to the parental cell, which allows direct comparisons between sexual and asexual strains without the potentially confounding effects of different genetic backgrounds or the use of a (temporarily) different environment to induce sexual sporulation. In one study[48], the sexual populations showed faster adaptation relative to the asexual populations when adapting to a raised temperature and osmolarity, but not in a similar but more benign environment, in which no adaptation was observed. In another study[49], the same yeast strains, now as a mixture, were allowed to adapt to two contrasting environments, one being a test tube that contained minimal growth medium and the other being the much more variable environment of a mouse brain. When sex was induced, the sexual strain won the competition in the mouse brain but not in the test tube, despite the fact that it also showed general adaptation to this environment. These results indicate an advantage to sex during adaptation to variable or harsh environments.

Sources of negative linkage disequilibrium

Although empirical studies have provided some evidence that sexual recombination can increase the rate of adaptation, at least in some conditions, two fundamental questions remain unanswered about how such

Box 2 | **Empirical approaches for estimating epistasis**

Comparing fitness of constructed mutants with a known number of mutations
This is the most direct and often most powerful approach, because it avoids uncertainties about the genotypes that are involved[51,57,61,62–64,67]. It consists of the following experimental steps: measuring the individual fitness effects of two mutations (W_1 and W_2, respectively); combining both mutations in the same individual and quantifying the fitness of the double mutant (W_{12}); and estimating the epistasis coefficient, ε, as the difference between the observed fitness of the double mutant and the value that is expected under the multiplicative model (W_1W_2). Significant deviation from the multiplicative expectation is evidence for epistasis; the sign and value of the deviation quantify the sign and extent of epistasis that is involved. However, this approach requires having access to methods for moving alleles among individuals, either by crosses and selection of the mutant progeny, or by molecular techniques for gene replacement, such as phage-mediated transduction in *Escherichia coli*[63].

The statistical relationship between fitness and number of mutations
When the above approach is not feasible, an alternative is to analyse the form of the decline in fitness that is associated with an increase in mutational load. Different approaches have been used to increase mutational load, including treatment with increasing doses of chemical mutagens[54,115,116], increasing levels of inbreeding[56,66,117], increasing mutation rates[60,118], or increasing periods of random mutation accumulation using population bottlenecks or other conditions of reduced purifying selection[53,58–60,65]. In all cases, the duration of the process is assumed to be proportional to the number of mutations that are accumulated. Regardless of the method, a linear relationship between the logarithm of fitness and the dosage, inbreeding coefficient, mutation rate, or period of mutation accumulation implies no epistasis, a downward curved relationship reflects negative epistasis, and an upward curved relationship indicates positive epistasis.

Purifying selection
Selection against deleterious alleles.

Clonal interference
Competition between multiple beneficial mutations that have arisen in different individuals in an asexual population.

Isogenic
Genetically identical.

Fitness components
All the traits of an organism that contribute to its reproductive success.

Compensatory mutation
A mutation that has a beneficial effect that depends on the presence of a deleterious mutation at another locus.

Truncation selection
A form of selection such that individuals with a phenotype below or above a certain threshold have a relative fitness of zero or one, respectively.

advantages arise. First, do sexual populations accelerate adaptation due to release from interference between different beneficial mutations[11,13,16,18–20] or due to the liberation of beneficial mutations from genomes that carry harmful mutations[26]? This question remains largely uninvestigated, although the efficacy of the second process was shown in a study that used experimental *Drosophila melanogaster* populations[24]. Second, which of the two causes of $D < 0$, drift or negative epistasis, is more important for the evolutionary advantage of sex? It is on the second question that we now focus.

Negative epistasis. Negative epistasis has been the focus of empirical work for a longer period than drift, probably because of the generality of explanations that involve epistasis, the fact that models that rely on epistasis were better worked out theoretically, and the experimental tools (for example, molecular genetic tools) that are available for studying interactions among constructed mutations. Over the past decade, several methods for measuring epistasis (BOX 2) have been applied to a range of organisms, including digital organisms, viruses, plants and animals. With few exceptions[50–52], the mutations that were studied were deleterious. The results from these studies provide a mixed picture: some report prevailing $\varepsilon < 0$ (REFS 50,53–56), some show prevailing $\varepsilon > 0$ (REFS 51,52,57–61) and several show no or variable epistasis[62–67]. So, no general support for $\varepsilon < 0$ has been found.

The observed sign of epistasis (that is, positive or negative) varies not only among different organisms, but also among different combinations of mutations and different fitness components within the same organism, which further limits the generation of $D < 0$ and, consequently, the advantage of sex[68]. Moreover, even when prevailing $\varepsilon < 0$ is found, it has not always been in the narrow range that is required for it to favour the evolution of sex and recombination[4,12,68].

The results of the studies that are discussed above are also likely to be of variable reliability owing to problems with the experimental approaches used. For example, natural selection often biases results towards $\varepsilon > 0$, either by complicating the isolation of low-fitness individuals or by favouring compensatory mutations in them. In addition, components of fitness are measured rather than fitness itself. Furthermore, the number of mutations is poorly controlled in studies that rely on indirect measures, and the mutations that are studied are a non-random selection of all possible mutations; for example, several studies have used marker mutations with visible phenotypes or transposon insertions that knock out entire genes. Altogether, the current support for the presence of suitable amounts of $\varepsilon < 0$ to promote the evolution of sex is weak.

A better understanding of the mixed empirical evidence for $\varepsilon < 0$ might come from understanding the underlying causes of this particular form of epistasis. Negative epistasis, like fitness in general, is a function of both the genotype and the environment. One intuitive environmental or ecological explanation of $\varepsilon < 0$ is that it is caused by the density-dependent regulation of population size due to limiting resources, resulting in truncation selection[69,70]. This form of selection can be seen as an extreme form of negative epistasis, in which individuals that carry more than a certain number of deleterious mutations are unviable, whereas they have maximum fitness if they carry fewer mutations.

The view that $\varepsilon < 0$ is associated with conditions of high population density, such as stable environments in which populations reach their carrying capacities, is consistent with the preference of sexual species for such environments[2]. To relate $\varepsilon < 0$ to metabolic functions that are relevant for particular ecological conditions, Szathmáry studied a simple model of a linear metabolic pathway[71]. Deleterious mutations that affect different enzymes in the pathway showed positive epistasis when selection was for maximum flux, but negative epistasis when selection was for optimum flux or optimum metabolite concentration. By assuming that maximum flux and optimum flux or metabolite concentration are relevant under conditions of high- and low-resource availability, respectively, he concluded that $\varepsilon < 0$ is restricted to conditions of intense resource competition.

The MDH[27,28] states that, provided that the genomic mutation rate for each generation (U) is greater than one, sex would be beneficial by purging deleterious mutations if $\varepsilon < 0$. Kondrashov argued that with $U >> 1$, species with

Table 1 | **Recent estimates of the genomic mutation rate**

Species	Mutation rate	References
RNA viruses*		
Bacteriophage Qβ	6.5	124
Influenza A	≥1	124
Poliovirus	0.8	124
Tobacco mosaic virus	0.12	125
Vesicular stomatitis virus	1.2, 3.5	126,124
***DNA prokaryotes*‡**		
Bacteriophage λ	0.0038	124
Bacteriophage M13	0.0046	124
Bacteriophages T-even	0.004	124
Escherichia coli	0.0001, 0.0002, 0.0007, 0.0025	127,128,129,124
***Eukaryotes*§**		
Arabidopsis thaliana	0.05, 0.1	130,131
Caenorhabditis elegans	0.0026, 0.008, 0.018, 1.68	132,133,124,134
Daphnia pulicaria	0.73	135
Drosophila spp	0.005, 0.058, 0.15, 0.35, 0.42, 0.5, 0.058, 0.070, 0.071	136,124,137,138,139, 140,140,140,140
Homo sapiens	0.16, 1.6, 3, 58, 80	124,141,140,142,143
Mus musculus	0.49, 0.5	124,140
Neurospora crassa	0.0030	124
Saccharomyces cerevisiae	0.0027	124
Triticum durum	0.04	144

Estimates are for the number of mutations at each generation per haploid genome, obtained for a wide variety of organisms and methodologies. *Median = 1. ‡Median = 0.004. §Median = 0.058.

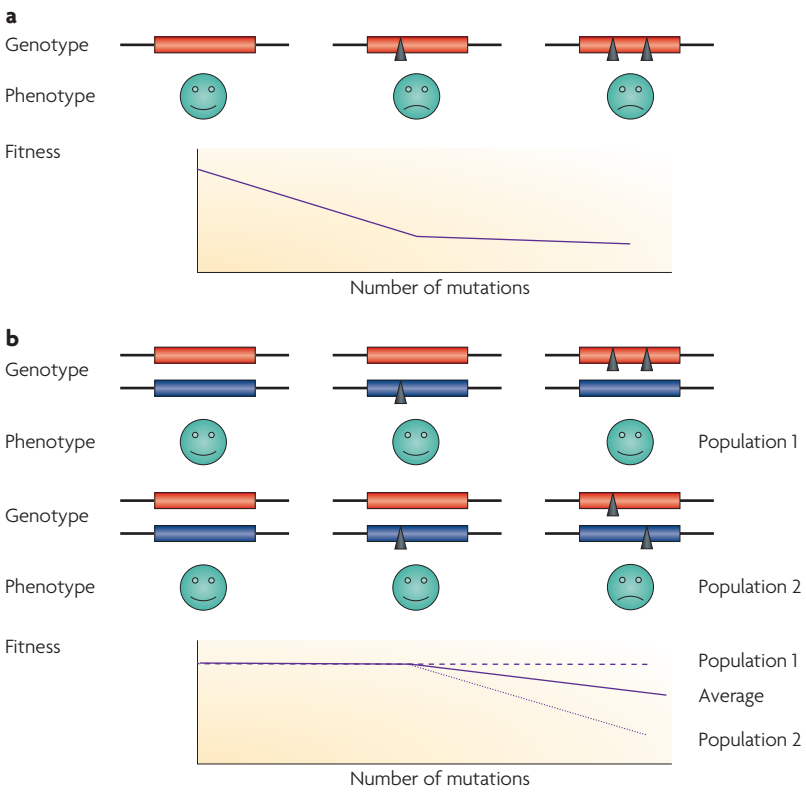

Figure 2 | **The relationship between robustness, redundancy and epistasis.** Two hypothetical genetic systems are shown; one is genetically redundant (panel **b**), and the other is not (panel **a**). Genetic units are indicated by rectangles, phenotypes are indicated by green faces and mutations are indicated by black triangles. The graphs show the fitness of the organism, which is the result of averaging the phenotypic effects of mutations across all the genetic elements of each system. **a** | In a non-robust system with no backup for the phenotypic property, the first mutation strongly impairs the phenotype; as a result, subsequent mutations no longer do much harm, which results in positive epistasis ($\varepsilon > 0$). **b** | In a redundant system with parallel genetic units that contribute independently to the same phenotype (red and blue rectangles), mutations have initially mild effects on fitness. However, as the number of mutations increases, all redundant elements will eventually be damaged by mutations and the function eventually collapses, resulting in negative epistasis ($\varepsilon < 0$). How fast the function declines depends on whether the second mutation hits in the same (shown as population 1) or the alternative (shown as population 2) genetic unit.

moderate population sizes could survive the damage of mutation accumulation only if mutations show $\varepsilon < 0$; so, finding $U >> 1$ is indirect evidence for $\varepsilon < 0$ (REF. 72). In principle, measuring U is easier than measuring epistasis, because U is a scalar parameter that is little affected by environmental changes and fundamentally depends on the efficiency of the replication machinery, whereas epistasis estimates depend on fitness, which can vary across environments. Whether U is greater or smaller than one is controversial, as shown by the variable estimates of U, sometimes even for the same organism (TABLE 1). According to the available estimates, only RNA viruses and a few higher eukaryotes (for example, humans) show $U > 1$. Moreover, according to the MDH, these estimates of U indicate that $\varepsilon < 0$ should exist in RNA viruses and not in DNA-based organisms, but this expectation is contradicted by the available evidence showing that $\varepsilon > 0$ is the rule for RNA viruses[51,58,59,61].

Deme
A local population of organisms of a sexual species that actively interbreed with one another and share a distinct gene pool.

Drift. Frustrated by the lack of evidence for $\varepsilon < 0$, researchers have recently taken new avenues to look for causes of $D < 0$. Theoretical studies indicate that drift combined with directional selection can generate $D < 0$, provided that populations are of intermediate size[73] or subdivided into demes[74], and that epistasis, irrespective of its sign, is weak[73]. If drift is to generate $D < 0$ between beneficial alleles, multiple beneficial mutations must occur, which is less likely for very small populations[16,18,19,73,75]. A recent theoretical study[76] shows that deleterious mutations at other sites can severely reduce the effective population size at a linked locus that is under selection (the background selection hypothesis[23–25]), and so increase the potential for drift to generate $D < 0$, even in very large populations. More generally, the advantage of sex in releasing populations from the adaptive constraints of clonal interference ($D < 0$ between beneficial alleles) also depends on the number and fitness effects of beneficial mutations, and might be smaller than previously thought[77]. Nonetheless, two empirical studies that are mentioned above support the significance of drift, which, combined with selection, causes clonal interference and provides an advantage of sex in adapting populations of *C. reinhardtii*[45] and ϕ6 (REF. 47). Last, it should be noted that a significant role for drift does not preclude $\varepsilon < 0$ as a contributing factor.

Pluralist approaches

Prompted by the inability to find a single universal advantage of sex, a growing number of studies are looking for sex benefits that arise from combinations of mechanisms. These so-called 'pluralist approaches'[78], which assume that either different mechanisms are responsible in different species or different mechanisms are combined in the same species, do not help to reduce the number of possible mechanisms, but can isolate combinations that work together to provide a sex advantage. Several recent empirical studies have looked at combinations of mechanisms that operate in the same species by studying the combined effect of environmental stress and mutation on fitness. Increased mutational load has been proposed to extend the parameter space in which sex is advantageous if Muller's ratchet is operating in conjunction with co-evolutionary dynamics that are caused by parasites[31,78,79]. Using parasites as the source of stress, aggravated fitness costs of mutations were found in *Escherichia coli*[80], *Pseudomonas fluorescens*[81], beetles[82] and sheep[83], but not in *Daphnia magna*[56,84]. These results are consistent with observations that have been made for other environmental stresses that increase the severity of deleterious mutations in yeast[85], *C. elegans*[86], and *D. melanogaster*[87], although variable effects were observed in *E. coli*[88].

The effect of parasites or other sources of environmental stress on the ratchet is twofold. If stress increases the average severity of mutations, the selective removal of mutations is facilitated and the ratchet should click more slowly[22], reducing the advantage of sex. However, if the effect of stress on mutational effects is not homogeneous but a subset of deleterious mutations is disproportionately aggravated (for example, see REF. 80), the rate at which

Box 3 | **The evolution of genome complexity**

Sex and recombination are not the only evolutionary causes of robust and modular genome architecture. One necessary condition for the evolution of robustness through redundancy is that genomes become more complex (for example, by containing more genes). Recent studies of the evolution of genome architecture that make use of the increasing availability of genome data point at a crucial role for genetic drift in the origin of genome complexity[119,120]. An important route towards increased genome size is gene duplication, which causes an immediate, albeit transient, genetic redundancy. The evolutionary fate of duplicated gene pairs is uncertain but diverse: in the majority of cases, one copy will be silenced by the accumulation of mutations, although in some cases new functions evolve and previous functions are lost[119,121]. One of the two gene copies might evolve a new function through neofunctionalization, that is, the acquisition of a new beneficial mutation in one copy, whereas the other retains the old function[122]. However, this process is thought to be rare, especially in small populations in which drift is more effective than selection[119]. A more likely process in small populations seems to be subfunctionalization, in which complementary subfunctions of both members of a gene pair are lost due to mutation, so that their joint expression becomes necessary to perform the ancestral functions. In this way, gene duplication and subfunctionalization convert single genes with multiple functions into multiple genes with fewer functions[119], resulting in genomic modules that are involved in the same function. The increase in genome size causes organisms to become larger and more complex, resulting in smaller effective population sizes, which enhances the role of drift in the origin of genome complexity[119,123]. This then sets the stage for adaptive processes, for example, involving sex and recombination, to further shape genome architecture in ways that are discussed in the text.

the ratchet clicks might be accelerated in the absence of recombination. This is because mild mutations accumulate more rapidly in the presence of other strongly deleterious mutations that reduce the effective population size and, consequently, intensify drift[89].

A few studies have looked at the effect of stress on the form of epistasis, again with mixed results. Cooper *et al.* found no evidence for $\varepsilon < 0$ among transposon-insertion mutations in either parasitized or parasite-free *E. coli*[80]. However, Killick *et al.* found $\varepsilon < 0$ among chemically induced mutations in *D. magna*, although only in the presence of particular bacterial endoparasite genotypes and at a particular temperature[90]. So, we currently have no evidence that $\varepsilon < 0$ would in general be promoted in the presence of biotic or abiotic stress. From their findings in *E. coli* that some stresses alleviated the negative effects of mutations, Kishony and Leibler inferred that epistasis must necessarily be negative in stressful environments and positive in favourable environments, because otherwise, highly mutated genotypes would replicate faster under stressful than favourable conditions[88]. Finally, using a computer model of the intracellular replication of bacteriophage T7, You and Yin found that mildly deleterious mutations interacted synergistically in resource-poor environments, but showed $\varepsilon > 0$ in a resource-rich environment[91].

Sex and genetic architecture

Recently, a new window has been opened in the hunt for conditions in which sex is likely to provide an advantage, by studying genetic architecture — the interactions between gene functions that shape phenotype. Studies of genetic robustness and evolvability have proved particularly interesting, and indicate that there is a two-way relationship between sex and genetic architecture. As a result, sex might even promote its own maintenance once it has arisen.

Genetic robustness as a cause of negative epistasis. Are there phenotypic features that imply negative epistasis and therefore promote the evolution of sex? Clearly, answers to this question should come from the way that genes cooperate to perform their biochemical, physiological and organismal functions — the genetic architecture of fitness. A model of yeast metabolism showed that epistasis is more common among genes that are involved in the same metabolic function, but the sign of epistasis varied among functions[92].

One feature that causes $\varepsilon < 0$ is genetic robustness — the stability of phenotypes in the presence of genetic perturbations such as mutation or recombination[93,94]. Many different mechanisms can cause genetic robustness, of which at least two lead to $\varepsilon < 0$: genetic redundancy and specialized buffering mechanisms such as chaperone molecules. Genetic redundancy is the situation in which more than one gene or pathway perform a similar function. This means that deleterious mutations have little effect until all pathways are affected by mutations, at which point fitness declines substantially[92,95] (FIG. 2). A recent *in vitro* study of a β-lactamase[96] showed that functional redundancy might even exist within proteins, leading to a stability threshold that buffers the deleterious effect of mutations on fitness, resulting in negative epistasis. Similarly, chaperones assist the proper folding of other enzymes and are thought to buffer deleterious mutations up to a certain number, after which the buffered function breaks down[97,98] (if chaperone expression is induced only after the accumulation of multiple mutations, apparent $\varepsilon > 0$ can result instead[60], because the effect of the first mutations would not be effectively masked). Other robustness mechanisms exist, such as distributed robustness of metabolic networks or modular genome architecture[94,99,100], but it is unclear whether these also lead to $\varepsilon < 0$.

Mutational robustness comes with at least two costs: first, it leads to the accumulation of mutations in the long term, and second, robustness mechanisms are energetically costly. A recent theoretical study shows that these costs can be compensated in sexual populations with intermediate recombination rates[101]. By breaking down the linkage between robustness genes and the target of the robustness mechanisms, recombination de-couples the short-term benefit of robustness (that is, enhanced fitness of mutant genotypes) from the long-term cost of mutation accumulation[101].

Sex influences genetic architecture. Sex itself might have affected genetic architecture and epistasis, perhaps in ways that bolstered its own evolution. It has been known for thirty years that recombination can affect the amount and strength of epistasis. In a bacteriophage T4 study in 1977, resistance to proflavine was shown to evolve as a result of a significantly larger number of genetic interactions in populations with low recombination compared with those with high recombination, with epistasis being

Robustness
The invariance of a phenotype or fitness in the face of genetic perturbations (for example, mutations) or environmental perturbations.

Evolvability
The long-term ability of a population or lineage to evolve new adaptive changes and prevent extinction.

Modularity
A specific form of genome architecture in which organismal or sub-organismal functions are encoded by discrete, contiguous parts of the genome.

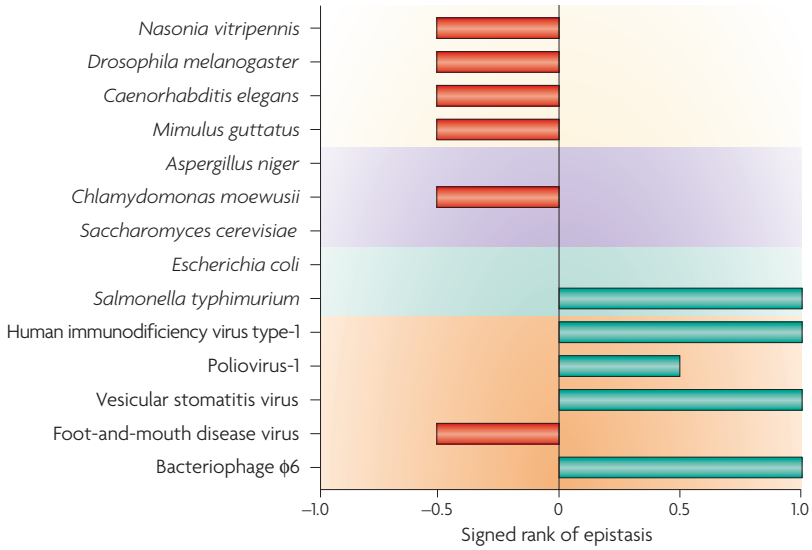

Figure 3 | **The relationship between epistasis and genome complexity.** Sanjuán and Elena[107] compiled information from 21 studies that used diverse methodological approaches to look for epistasis in fitness traits. The organisms ranged from RNA viruses to pluricellular eukaryotes. The result from each study was recoded as follows: a value of –1 was assigned when significant negative epistasis was observed, –0.5 for non-significant negative epistasis, 0 when multiplicative effects were obtained, 0.5 for non-significant positive epistasis, and 1 for significant positive epistasis. Here the median value for each organism has been plotted. Most cases that reported positive epistasis correspond to organisms with simple genomes (RNA viruses), whereas cases of negative epistasis are associated with increased genome complexity (eukaryotes). Coloured backgrounds indicate groupings of RNA viruses (shown in orange), bacteria (shown in green), unicellular eukaryotes (shown in purple), and pluricellular eukaryotes (shown in yellow).

on average more antagonistic in the low-recombination populations than in the high recombination populations[102]. However, because $\varepsilon > 0$ in both lines, but without a shift in the epistasis sign in the high-recombination lines, no advantage to sex was provided through $D < 0$.

For sex to benefit from epistasis, its sign has to be negative. Are there cases in which sex produces this type of epistasis? In this respect, the causal link between genetic robustness and $\varepsilon < 0$ might be important. Theory predicts that sex facilitates the evolution of genetic robustness, because sexual organisms are selected for their ability to perform under conditions of greater genetic perturbations (caused by recombination) than asexuals[93,101,103]. Two recent studies have now found support for this prediction. One used an artificial gene-network model, simulating the mutual interactions of four developmental genes in *D. melanogaster* to study the effect of recombination on robustness and epistasis[104]. Sex indeed increased the genetic robustness of the evolved networks, and $\varepsilon < 0$ evolved as a by-product, particularly if the network allowed numerous genetic interactions and the mutation rate was high.

A second recent study used digital organisms to test how sex reshapes genetic architecture[105]. Again, sex increased genetic robustness and changed the form of epistasis. However, sex only diminished the strength of $\varepsilon > 0$ (as in T4 (REF. 102) and T7 (REF. 91) bacteriophages), but did not cause epistasis to become negative. Interestingly, independent of its effects on epistasis, sex also increased the modularity of genome organization. Yeast genomic data indicate a possible mechanism for the modular organization of sexual genomes[92] in which there is selection for low recombination in regions that contain functionally related genes that must be maintained in clusters due to their shared regulation[106].

So, robustness and modularity can evolve as a consequence of sex, although there is a mixed picture for the epistasis that is associated with these changes. The fact that even infrequent recombination might affect genome organization over the long term complicates investigations of this issue and adds to the general problems with measuring epistasis. Furthermore, possible effects of sex and recombination might be confounded by other factors that affect epistasis, such as genome complexity, which could be correlated with robustness and modularity[101] (BOX 3). Indeed, a recent meta-analysis showed a significant trend towards $\varepsilon < 0$ with increasing genome size[107] (FIG. 3).

A final possibility is that sex, by making genomes more robust and modular, benefits from consequences other than the generation of $\varepsilon < 0$. Modularity and robustness are also thought to enhance the long-term evolvability of populations. A modular organization of genomes allows modules to be reused for new functions, especially in sexuals, in which recombination helps to try out new combinations of modules and reduces pleiotropic constraints, so that functions can be optimized individually and selective interference is avoided[99,108–110]. Genetic robustness might enhance evolvability by allowing the accumulation of genetic variation that is expressed only after the break-down of robustness, for example, due to a particular mutation or environmental perturbation[97,111] or by buffering the negatively pleiotropic effects of adaptations[109,112].

Conclusions and future perspectives

Substantial recent progress, from both theoretical and empirical work, has been made towards understanding the evolution of sex, providing a clearer picture of the possible mechanisms by which sex might provide an advantage, and the conditions on which these advantages depend. However, the picture is still incomplete. Increasing evidence suggests that sex speeds up adaptation, but whether $\varepsilon < 0$ or drift is central to this advantage remains unclear. Studies looking for $\varepsilon < 0$ under conditions in which such epistasis is predicted by ecological or genetic theories, such as intense resource competition or high levels of genome complexity, might help to solve problems with interpreting the existing data. As highlighted by recent theoretical work, the role of drift might have been a crucial source of $D < 0$ instead of, or as well as, $\varepsilon < 0$; distinguishing between these two mechanisms will be an important goal for future experimental work.

Finally, recent studies have indicated a two-way relationship between sex and genetic architecture. Sex and recombination cause genomes to become more modular and robust, with recombination possibly leading to negative epistasis. Furthermore, they might contribute

Pleiotropic constraint
Evolutionary constraint that is caused when a gene that is under selection due to a positive effect on one trait has simultaneous negative side effects on other traits, which limit or prevent its selection.

to increased evolvability and therefore provide a long-term evolutionary advantage[100,109–111]. Sex and recombination might in this way have forged their own evolution by reshaping genome organization to provide short- or long-term evolutionary advantages. Drift also seems to have been important in the evolution of genome complexity, from which sex might have benefited through increased long-term evolvability. A future challenge will be to distinguish between the relatively short-term benefits of sex that result from negative epistasis or drift, and the long-term benefits that might be associated with increased evolvability. Therefore, new empirical studies should, among other approaches, aim to understand the two-way relationship between sex and genetic architecture. In particular, the effect of recombination on genome organization should be further explored. Because these effects are predicted to be slow and visible only in the long term, experimental studies are only feasible in systems that allow rapid evolution and extensive manipulation of mutation and recombination rates, such as digital organisms or *in vitro* evolution of small genomes or even single enzymes. The relative simplicity of such systems allows the study of the two-way relationship between sex and genetic architecture in a way that allows genetic and phenotypic changes to be correlated. Comparative studies using genomic data from related species might also be informative owing to their ability to look far back into evolutionary time, providing further insights into the role of genome organization in the evolution of sex.

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Competing interests statement
The authors declare no competing financial interests.

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