EXPERIMENTAL TESTS OF THE ADAPTIVE SIGNIFICANCE OF SEXUAL RECOMBINATION

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Numerous theories have been proposed to explain the advantages of sexual recombination — the exchange of hereditary material between different genomes or homologous chromosomes. Many of these candidate benefits have been evaluated in controlled laboratory experiments, which, collectively, strongly indicate that sexual recombination provides important long-term advantages.

EVOLUTION OF SEX

BDELLOID ROTIFERS
Microscopic organisms that seem to have experienced a period of evolution without sex, and probably without other forms of recombination, for more than 80 million years.

The wide phylogenetic and geographical patterns of sexual recombination indicate that it has intrinsic advantages and, indeed, identifying these benefits has been the subject of long-standing theoretical and experimental studies. Any satisfactory explanation for why sex evolved and is maintained, however, must account for the intrinsic and substantial disadvantages that are associated with sexual recombination. These detriments include the twofold 'cost of producing males', which refers to the reduction in the intrinsic growth rate of a sexual population when males do not provide resources that increase the fecundity of their mates[1]; the twofold 'cost of meiosis', which reduces parent–offspring relatedness from 1, in a female that reproduces parthogenetically, to 0.5 in a sexually reproducing female[2]; and the break-up of co-adapted gene combinations[3].

Although recombination has countervailing advantages, recombining species have not totally outcompeted asexual, clonally reproducing lineages. In fact, asexual lineages are found among most of the main plant and animal groups[4–6]. Their success is shown by their persistence for thousands of generations and geographical distributions that frequently far exceed those of their sexual progenitors[4–6]. Despite this, most asexual lineages of plants and animals are derived only recently from sexual ancestors, and are therefore regarded as evolutionary dead-ends that do not persist over geological time — that is, for millions of years[1,5] (FIG. 1). The BDELLOID ROTIFERS[7], and possibly a few other small invertebrates[8,9], seem to be rare exceptions to this pattern, having persisted as asexual lineages for millions of years (see the accompanying article by Roger Butlin on page 311 of this issue). At the other extreme, some groups, such as birds and mammals, completely lack asexual lineages[5]. Because recombining lineages have adapted to aeons of sexual reproduction, the transition to proficient, asexual reproduction might be difficult to evolve[1]. Nonetheless, factors such as hybridization have apparently produced instantaneous asexual species with high competitive ability[4,5].

Like recombining species, genes that are located on recombining chromosomes persist over geological time, whereas most of those located on non-recombining Y chromosomes or organelle genomes do not[10–12]. So, just as there are rogue, ancient asexual species, there are also some non-recombining genes that have persisted over geological time[13].

Collectively, these patterns indicate that recombination is advantageous, but not universally essential. The observation that asexual species frequently outnumber their sexual progenitors and persist for thousands of generations[1,4–6] indicates that recombination frequently provides a long-term, rather than an immediate, advantage. Nonetheless, theory indicates that recombination can provide both short-term and long-term advantages (see the accompanying review by Otto and Lenormand on page 252 of this issue).

Below, I first identify which of the theoretical advantages to recombination are directly relevant to the

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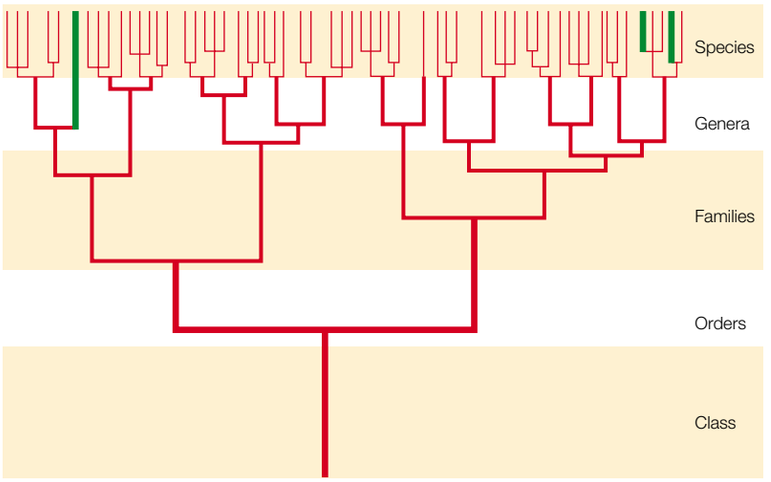

Figure 1 | **Typical phylogenetic distribution of asexual species.** The figure represents a schematic of a typical animal phylogeny. Asexual species (green) are rare (<0.1% of all animal species) and their lineages are short lived on a geological timescale. With a single exception (the bdelloid rotifers; see main text), no genus of substantial size, or any higher taxonomic group, is composed entirely of asexual lineages[5].

experiments that have been carried out so far. I then describe how this theory has been experimentally evaluated with controlled laboratory experiments.

Theoretical advantages of recombination

Evolutionary theories for the adaptive significance of recombination can be classified in many ways, but here I focus on two main types. Ecological theories are based on extrinsic factors that incorporate specific environmental or demographic contexts. For example, the pathogen ratchet theory[14] predicts an advantage to sex when recombination reduces the similarity in genetically encoded resistance factors between parents and offspring that are spatially clustered, and thereby reduces pathogen transmission between parent and offspring. By contrast, genetic theories, such as MUTATIONAL LOAD, derive from intrinsic hereditary factors, such as the mutation rate[15–18]. Because the same phylogenetic patterns that pertain to recombination are seen at the level of genes in genomes and of species in communities, as described above, and because the ecological theories apply only to the latter, the genetic theories describe the universal benefits to sexual recombination and I focus exclusively on them here.

There are two main experimental approaches to investigating the genetic advantage of recombination. One of these is to understand the causes of variation in recombination rate among genomes and genomic regions. This is an important aspect of the adaptive significance of sex, and involves modelling the evolution of genes that increase or decrease the amount of recombination within a genome (for more on this 'recombination-modifiers' approach, see the review by Otto and Lenormand on page 252 in this issue). However, the relevant experiments are limited to broad-scale measures of the rate of recombination observed in populations that have been subject to intense selection, rather than directly tracking the joint evolution of specific genes that influence fitness and modifier loci that mediate recombination between them. The relevant studies have been briefly reviewed elsewhere[19]. The second, 'competition-among-lineages' approach, contrasts the competitive fitness of closely related sexual and asexual lineages. Virtually all extant species recombine during at least part of their life cycle, and many of these have produced asexual lineages that could potentially displace their sexual progenitors[4–6]. Because most of the experiments that have addressed the genetic advantages of recombination concern competition between asexual lineages and their sexual progenitors, this will be my focus here. Before reviewing these experiments, I outline the theoretical advantages to recombination that they test.

Mutational load. One of the main theoretical advantages to recombination concerns its ability to reduce the mutational load[15–18], which is defined as the reduction in the fitness of a population due to the accumulation of deleterious mutations. Sexual lineages would be expected to out-compete asexual lineages, all else being equal, if their load of harmful mutations was found to be smaller. However, all else is not equal. As described below, the presence of males in sexual lineages can lead to ANTAGONISTIC COEVOLUTION between the sexes, SEXUALLY ANTAGONISTIC FITNESS VARIATION and an elevated mutation rate — all of which reduce female productivity and hence the competitive ability of sexual lineages. Sexual populations also accrue a higher burden of transposable elements[20–21]. These factors make it difficult to translate standard indices of mutational load (based on the mutation rate alone) into competitive exclusion between sexual and asexual lineages. Nonetheless, when the load of mutations is too high to be sustained by an asexual lineage, whereas it can be tolerated by a sexual lineage, then this can be attributed to an unequivocal advantage to recombination.

A second problem with traditional measures of mutational load occurs because they are quantified in the currency of mean fitness relative to a hypothetical, mutation-free genotype that is unlikely to occur in natural populations — that is, relative to an undefined and unmeasurable standard. The mutational load for an asexual population can be determined, however, by measuring fitness relative to the most fit genotype that is actually present in a finite population (BOX 1). This load becomes intolerable when the net reproductive rate of the fittest class in the population cannot compensate for it, leading to a progressive loss of fitness. The REQUISITE MUTATIONAL LOAD defines the maximum mutation rate that prevents the open-ended accumulation of mutations (BOX 2).

The requisite mutational load is decreased in recombining populations when there is positive assortative mating for fitness and/or an increase in the harmful effect of a mutation when other deleterious mutations are simultaneously present in a genome (negative epistasis, also referred to as synergistic epistasis, BOX 2). The efficiency of selection in eliminating deleterious mutations is increased by negative epistasis because it increases the number of deleterious

MUTATIONAL LOAD
The fitness reduction of a population owing to accumulated deleterious mutations in the gene pool.

ANTAGONISTIC COEVOLUTION
A cycle of adaptation and counter-adaptation between males and females of the same species or between a species and its enemies.

SEXUALLY ANTAGONISTIC FITNESS VARIATION
Variation in polymorphic genes that increase the fitness of one sex but decrease the fitness of the other sex.

REQUISITE MUTATIONAL LOAD
The excess in the net reproductive rate of the fittest class, above exact replacement, that is required to prevent open-ended mutation accumulation.

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Box 1 | Calculating the mutational load for an asexual population

The mutational load for an asexual population is determined by setting the relative fitness of the fittest extant genotype to 1.0 and solving for the equilibrium mean fitness (W_{mean}) that produces a stable frequency distribution of fitness classes. This can be done by focusing on the frequency of the fittest class ($Freq_{best}$)[15]. The relative fitness of the fittest class is defined to be $W_{best} = 1.0$ and the proportion of offspring that are not newly mutated is e^{-U}, because mutations are assumed to follow a Poisson distribution and e^{-U} is the proportional size of the zero class of a Poisson variate with mean = U mutations per genome per generation. The frequency of the best class increases each generation by reproduction and selection (that is, by a factor of W_{best}/W_{mean}) and decreases by new mutations that occur in some of its offspring (by a factor e^{-U}). The frequency of the fittest class across generations is defined by

$Freq_{best}{}^{*} = Freq_{best}$ (reproduction and selection) (proportion of unmutated offspring)
$= Freq_{best}\,(W_{best}/W_{mean})\,(e^{-U})$,

where (*) denotes the value in the next generation. Setting $W_{best} = 1.0$ and solving for equilibrium conditions (that is, setting $Freq_{best}{}^{*} = Freq_{best}$), gives $W_{mean} = e^{-U}$.

When is this load intolerable? We can answer this question by reformulating the measure of mutational load in the currency of absolute fitness — that is, in terms of the net reproductive rates of the various mutational classes. In this case, $W_{mean} = 1$ because, at equilibrium, each individual leaves exactly one offspring, on average. Solving for the fitness of the fittest mutational class extant in a population, $W_{best} = 1/e^{-U} = e^{U}$. If the fittest fitness class cannot achieve this net reproductive rate, then it will be lost from a finite population, as will be each successively next best class, and open-ended fitness decay will ensue. The maximum mutation rate that does not lead to deterministic, open-ended fitness decay is defined by the requisite mutational load[18].

mutations that are purged from the gene pool per selective death, and by positive assortative mating because it increases the variance in fitness among individuals. Recent theoretical work indicates that SEXUAL SELECTION, which is absent in asexual populations, might also reduce the requisite load of a sexual population[22–23]. This is because sexual selection among males will reduce the equilibrium frequency of harmful mutations in both sexes, but the cost of sexual selection is only experienced by males. Because males rarely contribute to the productivity of a population, and because sexual selection can be strong, the mutational load on sexual females could be substantially reduced.

Combining beneficial mutations. The second theoretical advantage to recombination is that it allows favourable mutations that arise in different lineages to be united in the same genome[3,24,25]. By contrast, in clonally reproducing species, different beneficial mutations must occur tandemly within the same lineage to come together in the same genome, and this slows the rate of accumulation of beneficial mutations (progressive evolution). A related theoretical advantage to sex also occurs when different beneficial mutations are present simultaneously in a population[26]. The rate of FIXATION of beneficial mutations that occur in the same asexual population is reduced through CLONAL INTERFERENCE, which puts a 'speed limit' on the rate of progressive evolution[27]. Clonal interference occurs because different beneficial mutations compete against each other, thereby diluting their advantage relative to the genomes that carry no beneficial mutations. Recombination re-assorts mutations that originate in different lineages (by reducing linkage disequilibrium), which allows selection to operate more independently on individual mutations and, in turn, causes each mutation to accumulate faster.

Background selection: general. A third theoretical advantage to recombination concerns the interaction between DIRECT SELECTION on a mutation and collateral selection on its genetic background(s). Deleterious mutations of small effect occur at a high rate in metazoans, and these frequently persist for several generations before being eliminated by natural selection[28–30]; recurrent mutation therefore causes gene pools to accumulate a burden of many mildly deleterious mutations. Variation in the mutational load among genomes generates a diverse spectrum of genetic backgrounds within which a mutation can arise. Variation in the number of beneficial mutations per genome also contributes to background selection, but deleterious mutations seem to be far more common and therefore are the predominant factor that causes background selection. The fixation or loss of new beneficial and deleterious mutations is strongly influenced by background selection, as described in the two following sections.

Background selection and beneficial mutations. Because a population must continually adapt to a changing environment, especially to coevolving competitors, pathogens, parasites and predators, there is an advantage to being able to incorporate efficiently a steady stream of new, favourable mutations. The fate of a new beneficial mutation depends on direct selection on the mutation itself, collateral selection on its genetic background and genetic drift (sampling error). Temporarily ignoring the effects of background selection, the probability of fixation of a beneficial mutation is approximately equal to $2s$, where s (the selection coefficient) is the increment by which the mutation increases fitness in the heterozygous state[3,31,32]. This approximation assumes that the selection coefficient is small and it ignores complicating factors such as epistatic interactions between genes, but it is a useful benchmark that is commonly used in evolutionary genetics. The probability of fixation of a beneficial mutation is less than one because mutations originate as single copies and are therefore susceptible to random loss by genetic drift until they accumulate to a substantial number: the larger the selection coefficient, the faster a mutation increases in number, and the smaller the cumulative probability of its loss by drift while it is rare.

The fate of new beneficial mutations also depends on their original genetic background and the presence or absence of recombination. Mutations that originate in high/low fitness genetic backgrounds have an increased/decreased probability of eventually fixing, owing to collateral selection on their background. When recombination is present, a mutation has only a transient association with its original genetic background, and this influence rapidly diminishes as a mutation

SEXUAL SELECTION
Competition among members of one sex (generally males) for fertilization opportunities with the other sex.

FIXATION
The accumulation of a mutation to a frequency of 100% in a gene pool.

CLONAL INTERFERENCE
The reduced competitive advantage of a clone that carries a beneficial mutation owing to the simultaneous presence of one or more other clones that carry different beneficial mutations.

DIRECT SELECTION
Darwinian selection on a specific mutation.

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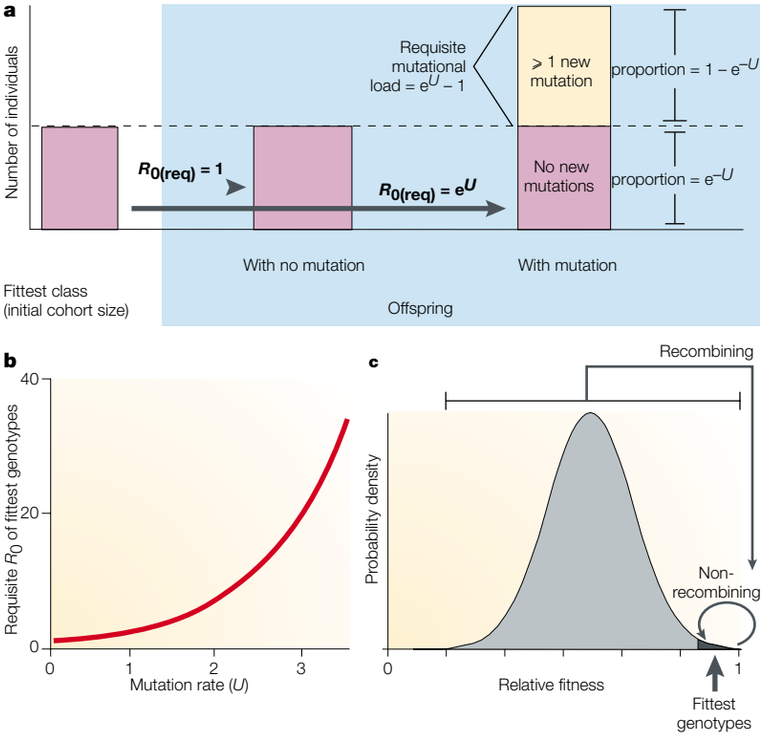
Box 2 | **Requisite mutational load**

Consider a finite population that is large enough to ignore sampling error. At equilibrium, the average fitness (as measured by the net reproductive rate, R_0 = average lifetime per-capita number of offspring) is equal to one. To prevent the open-ended erosion in mean fitness (defined here to be an intolerable mutational load), the distribution of fitness classes must be anchored such that, at equilibrium, the fittest class does not deterministically decline in frequency each generation. To be stable, the net reproductive rate of the fittest class must be one after discounting for offspring that are newly mutated and therefore lost from the fittest class (panel **a**). If the productivity of the fittest class is insufficient to offset recurrent mutation, it will be lost recurrently, causing an intolerable mutational load to accrue. Unlike MULLER'S RATCHET — in which mutations accumulate due to the stochastic loss of the fittest class — mutations, in this case, accumulate deterministically due to insufficient productivity of the fittest class relative to the loss by mutation.

To determine the productivity of the fittest class that is required to prevent an intolerable mutational load ($R_{0(req)}$), it is assumed that harmful mutations occur at rate U per genome per generation. Because there are a large number of mutable loci and a small independent probability of mutation at each locus, U is expected to follow an approximate Poisson distribution, and a fraction e^{-U} of offspring will be unmutated (e^{-U} is the fractional size of the zero class of a Poisson distribution). To compensate for the fraction of offspring that are newly mutated, $R_{0(req)}$ must be $1/e^{-U} = e^{U}$, so the requisite mutational load is $e^{U} - 1$ (panel **a**). $R_{0(req)}$ is not the maximum reproductive rate of the fittest genotype; it is the average per-capita number of offspring produced by the fittest type under competitive equilibrium conditions when an average individual produces only a single surviving offspring. $R_{0(req)}$ leads to extinction in an asexual population when U is sufficiently large relative to the realized growth rate of the fittest class (panel **b**). Panel **b** shows that the requisite load ($R_{0(req)} - 1$) increases exponentially with the genome-wide mutation rate.

With recombination, the fittest class is not produced by its own clonal reproduction, but by recombination among the population at large (panel **c**). When there is negative epistasis (that is, a mutation is more harmful when other harmful mutations are also present[14]) or positive assortative mating for fitness (that is, when genotypes of similar fitness mate predominantly among themselves[18]), then recombination reproduces the fittest class faster than it would have done by means of its own clonal reproduction. In this case, a recombining population can persist in which an asexual population would be destroyed by the open-ended accumulation of deleterious mutations — that is, by an intolerable mutational load.



recombines into new genetic backgrounds. Theoretical analysis[33–38] shows that, when recombination is present, the decline in frequency that a mutation would receive when it originates in an inferior genetic background is mostly compensated, on average, by the boost that it would receive when it originates in a superior genetic background (BOX 3, panel a). Therefore, for a beneficial mutation, recombination causes the average effect of background selection to be negative, but relatively small.

When recombination is absent, background selection can strongly decrease the average probability of fixation of a beneficial mutation[33–38] (BOX 3, panel b). In a non-recombining population, high-fitness genotypes gradually displace low-fitness genotypes, leading to recurrent selective sweeps by lineages from the highest end of the distribution of genetic backgrounds (the progenitor tail[38], BOX 3, panel b). As a selective sweep proceeds, deleterious mutations accumulate in the sweeping lineage(s) so that, at equilibrium, the distribution of fitness values does not change over time. These recurrent selective sweeps lead to the gradual extinction of all lineages that do not reside in the progenitor tail; hence, lineages outside the progenitor tail are collectively called the 'living dead'[38] (BOX 3, panel b).

When recombination is absent, mutations that originate in the living dead are trapped in their original genetic background and are doomed to eventual extinction unless their selection coefficient elevates the recipient genome into the progenitor tail. Beneficial mutations that reside in the progenitor tail, if not lost early on by sampling error or by competition among the fittest genotypes, will eventually fix in the population (BOX 3, panel b). Most new beneficial mutations, however, will be trapped in the living dead, and their loss due to background trapping causes them to accumulate far more slowly in a non-recombining population (FIG. 2). Background trapping will be an important cost whenever the heritable variance in fitness among genetic backgrounds is substantial relative to the selection coefficient (s) of a mutation (BOX 3, panels a,b).

So, in a non-recombining population of genomes or chromosomes, beneficial mutations are commonly trapped in the living dead (background trapping) and cannot become established in a population. Recombination frees beneficial mutations from their original genetic background, and thereby increases their probability of fixation.

Background selection and harmful mutations. Most mutations are harmful and one of the main functions of natural selection is to continuously purge these mutations from the gene pool. Deleterious mutations can accumulate (retrogressive evolution) by genetic drift when the strength of selection is small relative to random fluctuations in gene frequency due to sampling error. In the absence of complicating factors, such as tight linkage to other selected genes, genetic drift will overpower selection whenever $|s| < 1/N$, where N is the census size and s is the selection coefficient[32,39]. The probability of fixation of a neutral mutation due to genetic drift is $1/(2N)$, and this value

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MULLER'S RATCHET
Recurrent stochastic loss of the fittest genomes in an asexual population.

CENSUS SIZE
The total number of individuals in a population.

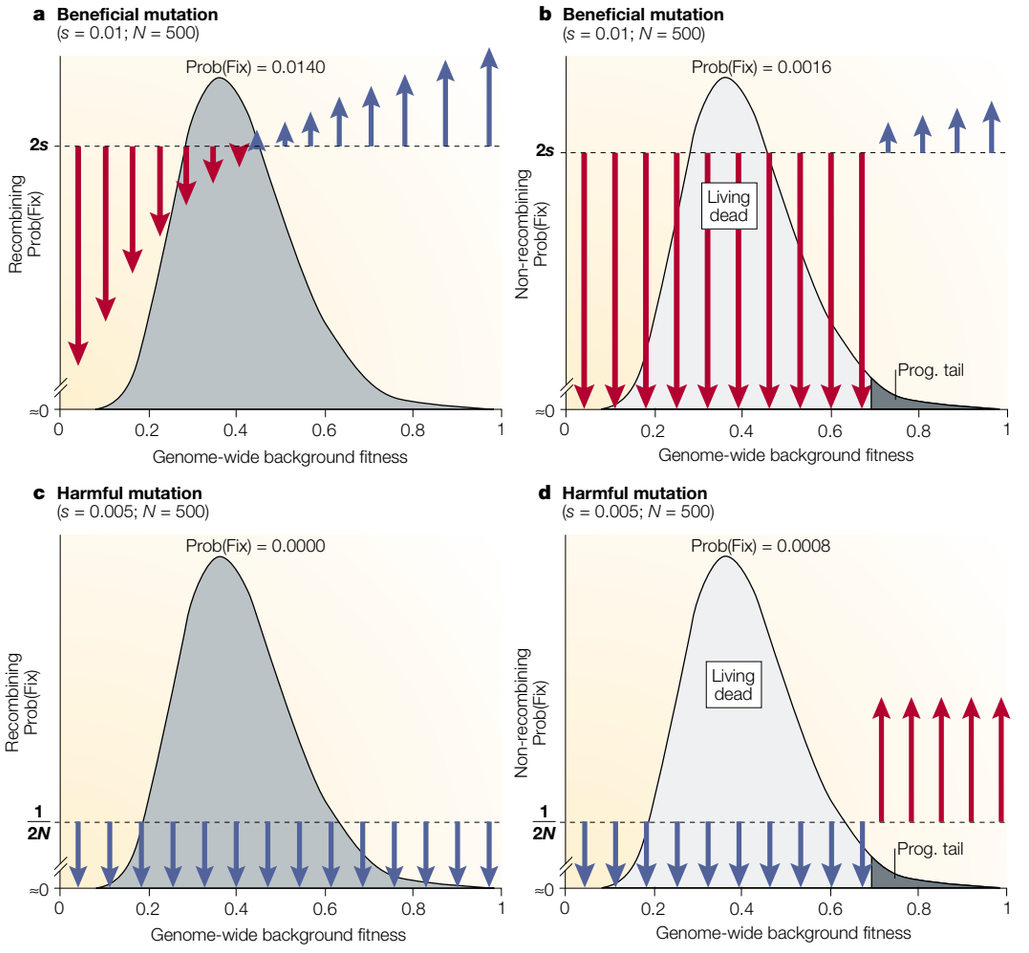
SELECTIVE SWEEP
The gradual accumulation to fixation of a genome or chromosomal region that has a net selective advantage.

Box 3 | Genetic backgrounds and fixation of mutations

The genetic background of a mutation (shaded fitness distribution) influences its probability of fixation (Prob(Fix); log scale) in a manner that depends on the presence or absence of recombination. As a benchmark for comparison, the probability of fixation in the absence of background selection is $2s$ for beneficial mutations (panels **a**, **b**) and maximally $1/(2N)$ for harmful mutations (panels **c**, **d**), where s is the selection coefficient and N is the CENSUS SIZE. Arrows depict the influence of the initial genetic background of a mutation on its probability of fixation: blue arrows reinforce natural selection and red arrows oppose it.

A beneficial mutation has a higher chance of being fixed in a recombining population (panel **a**) than in a non-recombining one (panel **b**). Recombination causes the original genetic background of a mutation to be transient and, because the influence of different starting backgrounds nearly cancel ($\sum$ arrows ≈ 0), there is only a small influence of background selection, on average. By contrast, in a non-recombining population, beneficial mutations are trapped in their genetic background of origin. A non-recombining population is functionally divided into a small 'progenitor tail' (Prog. tail), which is composed of genotypes (genetic backgrounds) that have the highest or nearly highest Darwinian fitness, and the 'living dead', which is composed of less fit genotypes — these are called the living dead because lineages of these genomes are destined to eventual extinction owing to selective sweeps of the progenitor tail. The probability of fixation of a beneficial mutation is lower in a non-recombining population because only a minority of these mutations that originate by chance in the progenitor tail can potentially accumulate to fixation; all others are trapped in the living dead and are destined to eventual loss.

Natural selection is sufficiently strong to prevent large-effect deleterious mutations ($|s| >> 1/N$) from accumulating to fixation in both recombining and non-recombining populations, but too weak to prevent the accumulation of small-effect deleterious mutations ($|s| < 1/N$). Deleterious mutations with intermediate effects ($1/N_p > |s| > 1/N$, where N_p is the size of the progenitor tail), however, accumulate only in non-recombining populations (shown here in panels **c** and **d**). Their selection coefficient is too large to allow their accumulation in a recombining population because selection is too strong relative to drift in the population at large (that is, $|s| > 1/N$). They can, however, accumulate to fixation by drift in the progenitor tail of a non-recombining population because drift is stronger in this smaller subpopulation (that is, $|s| < 1/N_p$) — in which case, they will eventually spread to the entire population through a SELECTIVE SWEEP. The patterns shown are general, but the calculations to produce the figures assume a coefficient of variation of 17% background fitness, and that the size of the progenitor class is 4% of the census size. Adapted from REFS 31–38.



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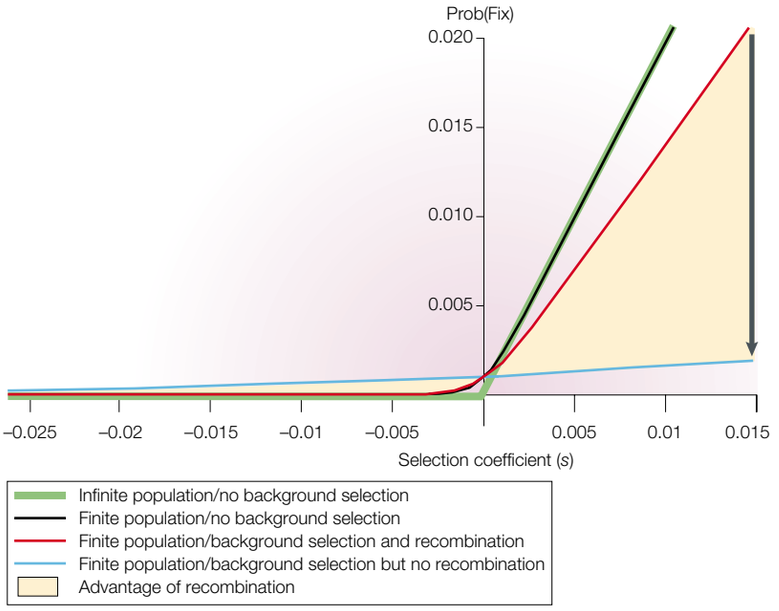

Figure 2 | **Fate of a mutation depends on direct selection (s), background selection and recombination.** The probability of fixation (Prob(Fix)) of a beneficial mutation is reduced by background selection, but more so when recombination is absent. The probability of fixation of a harmful mutation is increased by background selection, but to a greater degree when recombination is absent. The patterns shown are general, and the specific values shown on the graph are calculated as in BOX 3. Adapted from REFS 31–36.

is the upper bound for the fixation probability of deleterious mutations[31,32]. Factors such as fluctuations in population size and an unbalanced sex ratio generally make the EFFECTIVE POPULATION SIZE smaller than the census size. To avoid unnecessary detail here, I set the census size equal to the effective size and then evaluate the influence of recombination on the efficacy of natural selection.

In a recombining population, mutations freely move between genetic backgrounds, and deleterious mutations can accumulate only when $|s| < 1/N$ (BOX 3, panel c; FIG. 2). In a non-recombining population, most deleterious mutations originate in the living dead and they will be deterministically eliminated owing to their inferior genetic background (BOX 3, panel d). In this case, background selection reinforces direct selection on the mutation. Harmful mutations, however, also originate in the progenitor tail and these can accumulate by drift whenever $|s| < 1/N_p$, where N_p is the size of the progenitor tail (which is $<< N$, BOX 3, panel d; FIG. 2). So, harmful mutations with a very small effect ($|s| < 1/N$) accumulate in both recombining and non-recombining populations, those with small but intermediate effects can accumulate only in non-recombining populations ($1/N < |s| < 1/N_p$), and mutations with large effects ($|s| > 1/N_p$) will not accumulate irrespective of the presence or absence of recombination. FIGURE 2 summarizes the influence of background selection on beneficial and deleterious mutations. Although the advantage to recombination is larger for beneficial compared with harmful mutations (FIG. 2), most mutations are deleterious with small effects[28–30]. Accordingly, the potential advantage to recombination to prevent fixation of small-effect harmful mutations ($1/N < |s| < 1/N_p$) is substantial.

A final point concerning harmful mutations is the common misconception relating to retrogressive evolution in asexual populations, known as Muller's ratchet. It is frequently stated that Muller's ratchet occurs only in small non-recombining populations. This spurious conclusion is an artefact of the simplifying assumption that all harmful mutations have the same effects on fitness, set equal to the average effect of a deleterious mutation. When the effects of mutations are more appropriately assumed to be variable, with a large class of mutations that have a very small effect on fitness[29,30] ($|s| << 0.01$), then deleterious mutations will accumulate in populations of any finite size and the domain of Muller's ratchet is not restricted to small populations[40–43].

Experimental tests: mutational load

Theory predicts that asexual reproduction can persist only when the mutational load is tolerable. This will be true when the net reproductive rate of the fittest genotypes ($R_{0(best)}$) equals or exceeds the requisite net reproductive rate ($R_{0(req)}$) that is needed to offset recurrent deleterious mutations — that is, when $R_{0(best)} \geq R_{0(req)} = e^U$ (BOX 2). Experiments to test this prediction must evaluate both the genome-wide deleterious mutation rate (U) and the net reproductive rate of the fittest genotypes ($R_{0(best)}$). If recombination is to rescue a species from an intolerable mutational load, then there must be evidence for negative epistasis, positive assortative mating for fitness and/or compensating sexual selection, which give recombining species an advantage by increasing the power of natural selection to remove harmful mutations.

Requisite mutational load. Direct empirical estimates for $R_{0(best)}$ from natural populations are difficult to obtain because they require reliable measurements of the heritable lifetime fitness of the fittest genotypes under equilibrium conditions. This measure, however, was recently estimated from a high fecundity laboratory population of *Drosophila melanogaster*[44]. The population had adapted to a competitive laboratory environment for more than 200 generations. Despite the fact that females can lay more than100 eggs per day, the empirical estimate of $R_{0(best)}$ was only 1.82 — that is, the fittest female genotypes ($n = 40$) had a heritable net fitness that was only about twice as large as an average female. Although a survey of a larger number of genomes would be expected to find a larger estimate of $R_{0(best)}$, this value is still a useful first approximation. In these experiments, the fitness of genomic haplotypes was measured rather than the fitness of complete diplotypes. Extrapolating to diploid fitness, $R_{0(best)}$ is estimated to be ~3.3. Therefore, the maximum deleterious mutation rate that could be tolerated by this population, if asexual, would be $U_{max} = \ln(R_{0(best)} = 3.3) = 1.2$ mutations per genome per generation. These data indicate that the maximum tolerable mutation rate for a high fecundity species, such as *Drosophila*, would be $U_{max} \approx 1$. Because many species have mutation rates far in excess of this value, asexuality

EFFECTIVE POPULATION SIZE
The equivalent number of breeding adults in a population after adjusting for complicating factors such as nonrandom variation in family size or stochastic fluctuation in population size.

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would be predicted to lead to eventual extinction. However, comparable measures from different species in natural environments are needed to estimate, more generally, the maximal tolerable mutation rate.

Deleterious mutation rate. An evaluation of the requisite mutational load requires that we estimate the genome-wide deleterious mutation rate. This rate has been the subject of many recent reviews[45–49], so here I highlight only a few key studies. Most data to estimate the deleterious mutation rate come from mutation accumulation experiments. In these experiments, a population is recurrently bottlenecked to one or few individuals so that mutations of minor effect can freely accumulate by drift. The mathematical technique that is used to calculate the deleterious mutation rate from these experiments produces an estimate that is biased downwards in proportion to the coefficient of variation in fitness of mutations, which will be substantial when mutations vary in their impact on fitness and when there are many mutations of small effect.

Recently, an ingenious experiment was used to estimate the degree of this bias[23]. The mutation rate of *Caenorhabditis elegans* was manipulated by using a mutagen (ethyl methane sulphate, EMS) to produce a controlled genome-wide mutation rate of known minimal value. Populations of EMS-treated worms were subject to a mutation accumulation protocol and the mutation rate was estimated. Remarkably, almost all mutations (96%) were undetected. Statistical analysis indicated that most of the new deleterious mutations had a very small selection coefficient ($s << 1\%$). If most species have a large class of very small effect mutations, which seems likely[23,24], then the mutation accumulation procedure will grossly underestimate the genome-wide deleterious mutation rate.

An alternative to the mutation accumulation protocol is to estimate the deleterious mutation rate in species for which divergence time can be determined from the fossil record[50]. The sequence divergence of exons, after discounting by the projected number of neutral substitutions, is used to estimate the deleterious mutation rate. An extension of this technique was recently applied to a wide spectrum of species[22]. Deleterious mutation rates (adjusted for mutations in non-coding regions, transposon transpositions, and small insertions and deletions) increase linearly with generation time (0.34 for *Drosophila*, 1.1 for the laboratory mice and rats, 3.2 for the domestic dog and cat, and 6.6 for humans and chimps). At the lower end, these estimates would produce requisite mutational loads that — making feasible extrapolations from the available data — seem compatible with asexual reproduction, but not at the higher end. Kondrashov[48] has criticized these estimates (but see the rebuttle outlined in REF. 46), arguing that they are biased downwards but, nonetheless, they represent our best estimate of this contentious parameter.

Negative epistasis. As described above, for recombination to rescue a population from an intolerable mutational load there must be negative epistasis, positive assortative mating for fitness or compensating sexual selection. All of these processes increase the capacity of natural selection to purge harmful mutations from the genome, and thereby increase the productivity of a recombining species. This and the following two sections discuss the tests that have been done to detect the occurrence of any of them in experimental populations.

Many experiments have tested for negative epistasis (BOX 4). On balance, experimental support for the widespread occurrence of epistasis is inconsistent at best, and when detected, it was equally likely to promote as detract from an advantage to recombination. One criticism of past tests for negative epistasis is that they evaluate arbitrary spontaneous mutations[41,51]. Intrinsically interacting mutations, such as those that code for components of the same enzymatic or developmental pathways, will more plausibly produce negative epistasis[52]. Epistasis that is restricted to closely interacting genes is nearly as powerful in reducing mutational load as that between arbitrary mutations, but experimental tests for this kind of epistasis are lacking[18].

Positive assortative mating. Positive assortative mating for fitness is another powerful way to reduce the mutational load of a recombining population[18]. This mating system increases the variance in fitness among genotypes and thereby increases the HERITABILITY of fitness and the efficiency of selection. There are many natural history contexts that lead to positive assortative mating for fitness[53,54]. For example, in many species there is positive assortative mating for body size. To the extent that larger individuals achieved higher mass because their genotype made them better adapted to their ecological niche, then this mating pattern will produce positive assortative mating for fitness. Unfortunately, I found no experimental tests for the prevalence of this process in natural or laboratory populations.

Sexual selection. Lastly, sexual selection among males might reduce the mutational load of recombining species[42,43]. The critical assumption that remains to be tested experimentally is that sexual selection among males reduces the mutational load in females. The only direct experimental study that compared the load (measured by female productivity) of populations with and without the operation of sexual selection is an experiment with *D. melanogaster*[55]. This study found that the removal of sexual selection increased, rather than decreased, the productivity of females. The reduced load was due to reduced male-induced harm to their mates, which is expected to occur when males and females coevolve antagonistically[56–60].

Another potential countervailing cost of sexual selection is increased mutation rate. In species in which females have fewer mitoses per generation than males in their germ line (as in humans), empirical data indicates that males have a substantially elevated mutation rate (for example, fourfold higher in humans[61]), and this can substantially increase the mutational load of a sexual species[62].

HERITABILITY
The fraction of the phenotypic variance that is attributable to additive genetic variance

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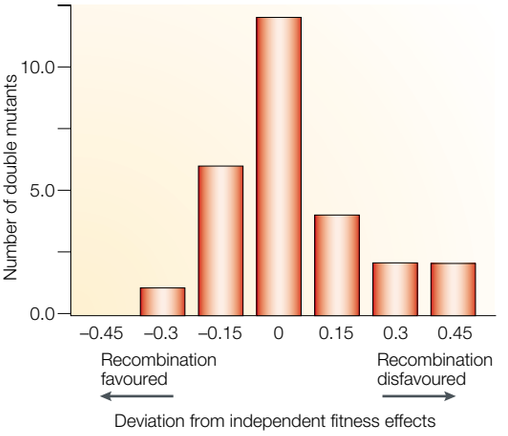
Box 4 | **Experimental tests of negative epistasis**



In one of the most elegant experiments so far, Elena and Lenski[80] inserted variable numbers of transposable elements into the genome of *Escherichia coli* and then measured net fitness. They found that fitness effects of the inserts typically combined independently (indicating little or no epistasis) and in the rarer cases when they did not, mutations were just as likely to combine in a way that favoured recombination (negative epistasis) as did not (positive epistasis) (see figure). The figure shows the distribution of deviations (observed–expected) in the fitness of doubly mutated *E. coli*. Expected values were calculated assuming no epistasis among mutations. A value of zero indicates no epistasis, positive values indicate positive epistasis and negative values indicate negative epistasis. The symmetry of the graph about zero indicates that there is no net advantage to recombination due to negative epistasis. (Data taken from REF. 80.)

A second experimental approach was to plot fitness against time for data collected from mutation accumulation lines. Although early studies with *Drosophila* that measured a fitness component (viability) found evidence for negative epistasis[81,82], this result was not confirmed by more recent experiments, with viruses, that measured total fitness[83,84].

A third approach, which had particularly high experimental power, measured fitness of new deleterious mutations in genetic backgrounds that had normal versus elevated numbers of deleterious mutations[85]. Data from this experiment do not support the common occurrence of negative epistasis. A fourth approach was to cross lines with different numbers of mutations and compare the fitness of parents and offspring. Data from this procedure seemed to support negative epistasis in *Chlamydomonas*[86], but this interpretation has been criticized[87]. In addition, a negative result was reported in a similar study with yeast[88].

Last, a study using *Drosophila* tested for negative epistasis among combinations of chromosomal regions that were marked with homozygous recessive, visible markers[51]. After adjusting for the fact that one of the marked regions increased fitness (rather than decreasing it as was originally expected because of the expression of the visible marker), this study found that most (36 out of 52) interactions between chromosomal regions were consistent with non-epistatic fitness interactions, and of those that were not, eight supported negative epistasis and eight supported positive epistasis.

For sexual selection to reduce the mutational load, there must be a positive genetic correlation for fitness between the sexes: genomes that produce high/low fitness males must also produce high/low fitness females. Some studies of sexual selection have found a correlation between the mating success of a male and the viability of his offspring[63,64]. However, the crucial parameter is the genetic correlation between sexual selection in males and productivity in females. Recently, the fitness of the same 40 cloned genomes was measured in both male and female *Drosophila*[44]. The intersexual genetic correlation for juvenile fitness (egg-to-adult viability) was positive. But in adults, in which gender roles diverge, the genetic correlation between male mating success and female fecundity was negative. These data indicated that adult males and females are selected towards different phenotypic optima and, because most genes are expressed in both sexes[65], selection in males leads to reduced female productivity (a phenomenon known as intersexual ontogenetic conflict)[44,66].

Additional data are needed to resolve the degree to which sexual selection influences the burden of mutations in females. The available experimental data indicates that elevated mutation rates in males[61,62], antagonistic co-evolution between the sexes[56–60] and sexually antagonistic fitness variation[44,66] will cause the operation of sexual selection to increase rather than decrease the mutational load of a population.

Experiments: combining beneficial mutations

There has been limited experimental evaluation of the hypothesis that recombination is favoured because it reduces interference between beneficial mutations that are simultaneously accumulating in a population. I found no experiments that introduced two or more beneficial mutations into populations with and without recombination, and then tested for faster production of genomes that carry compound mutations when recombination was present. However, the general concept that recombination speeds the rate of progressive evolution has been tested in other contexts. Experiments with *Escherichia coli*[67] and RNA viruses[68,69] have measured the rate of adaptation when a population is exposed to a new environment and compared this with a theoretical benchmark that assumes no clonal interference. There were two basic experimental designs. In the first, populations of different size were exposed to the same new environment and the rate of fitness increase was tracked over time. In the absence of clonal interference, the rate of adaptation should increase linearly with population size because the rate of production of new beneficial mutations is proportional to population size. In both experiments[67,68], the rate of adaptation levelled off as

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population size increased, supporting the operation of clonal interference. In the second experiment, the magnitude of the selection coefficient of the first mutation that was fixed in populations of varying size was compared. Clonal interference predicts that, on average, the selection coefficient of fixed mutations should increase with increasing population size, and this prediction was confirmed[69]. The rationale for the prediction is that, as population size increases, there will be more simultaneously competing beneficial mutations, so those with smaller selection coefficients will be competitively displaced. Although these experiments provide support for the operation of clonal interference, a more convincing case will be made when parallel experiments show that the harmful effects attributed to clonal interference are ameliorated or retained when recombination is present or absent.

Experimental tests: background selection

Recombination is predicted by theory to both slow the rate of accumulation of small-effect deleterious mutations and speed the accumulation of beneficial mutations. Both of these hypotheses have been experimentally evaluated, but most work has focused on beneficial mutations.

Harmful mutations. Few experiments have tested whether recombination slows the accumulation of minor-effect harmful mutations. Numerous experiments with bacteria and viruses showed that recurrently bottlenecking a population to a size of one haploid individual leads to fitness decline (many experiments are reviewed in REF. 70). These experiments, however, are more akin to traditional mutation accumulation studies (for example, REF. 81) rather than tests of the Muller's ratchet process. Bottlenecks to $N = 1$ haploid individuals cause mutations to be fixed and, therefore, mutations will accumulate irrespective of the presence or absence of recombination.

One line of experiments with an RNA bacteriophage [71,72] has taken the mutation accumulation protocol one step further by first bottlenecking populations 40 times and then measuring their fitness recovery with and without recombination. All populations recovered fitness rapidly (32% of fitness was recovered, on average, after eight growth cycles without bottlenecks), showing the substantial potential for beneficial and/or compensatory mutations to mitigate the accumulation of harmful mutations in a non-recombining lineage. In addition, when lines that had accumulated independent mutations were recombined, they recovered more rapidly (an additional 15% recovery of fitness).

A different approach was taken in experiments using a *D. melanogaster* model system[73]. Here, 80% of the genome was made to co-segregate like a giant non-recombining neo-Y sex chromosome or a recombining neo-X chromosome. The populations were maintained at an effective population size of 48 chromosomes each for 35 generations. At this small size, mildly deleterious mutations were expected to accumulate on both chromosome types, but at an accelerated rate in the absence of recombination. As predicted, the non-recombining neo-Y chromosomes degenerated rapidly, but recombination rescued the neo-X chromosomes from most of this fitness decay.

Beneficial mutations. A large number of experiments have tested the hypothesis that recombination speeds the rate of accumulation of beneficial mutations. Most of these experiments do not trace the fate of individual beneficial mutations, but instead measure the rate of progressive evolution with and without recombination. In one of the first, Carson selected motility behaviour in *Drosophila robusta* populations that either had no chromosomal inversions (which suppress recombination in heterozygotes) or had many inversions[74]. The populations with lower recombination had a trend towards a slower response to selection, but this difference was confounded by differences in the starting genetic variation among experimental treatments. McPhee and Robertson[75] extended this line of research by selecting for bristle number in populations of *D. melanogaster* with and without crossover among the autosomes (which constitute 80% of the genome). When crossover was present, the response to selection was 22–28% faster (18 out of 20 lines responded faster than the mean response when crossover was absent; $p < 0.01$, binomial test), supporting the hypothesis that recombination speeded the rate of accumulation of beneficial alleles that influence bristle number in the selected direction.

Markow[76] expanded this design by controlling crossover on both the X and the autosomes, so that 20, 40, 60, 80 or 100% of the genome was able to recombine. When she applied selection to phototaxis instead of bristle number, she reported that recombination significantly speeded the response to selection only in certain cases. To pool all her data, I regressed the response to selection on the percentage of the genome that recombined and found a highly significant positive correlation ($p = 0.0017$, $R^2 = 0.63$), indicating that as more of the genome was allowed to recombine, the response to selection was faster (W.R.R., unpublished observations). By contrast, Thompson[77] carried out a similar experiment (but controlled crossover only on the two main autosomes) and concluded that there was no significant effect of the presence or absence of recombination. My analysis of Thompson's data indicated a significant increase in the response to selection when recombination was present (Student's *t*-test, $p = 0.0163$; W.R.R. unpublished observations), but Thompson concluded that this difference was an artefact of the influence of the genetic constructs (balancer chromosomes) that were used to suppress recombination.

The next generation of experiments on the adaptive significance of recombination used bacteria, yeast, bacteriophage and viruses as model systems. These systems have the advantage of fast generation time, but the disadvantage of small genome size (that is, low background selection) and, hence, the advantage of recombination is expected to be smaller than it would be in metazoans with larger genomes (BOX 5).

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Box 5 | **Recombination and adaptive evolution in microorganisms?**

In 1977, Malmberg[89] adapted the bacteriophage T4 to a novel environment (proflavine in the medium) and experimentally controlled the level of recombination (low versus moderate). His data indicated that the phage adapted more rapidly when recombination was higher. The only complication with these paradigm-setting experiments was that the results are expressed as deviations from control populations, and in some cases the benefit attributed to recombination was associated with changes in the controls.

The next approach with microorganisms used *Escherichia coli* as a model system. Souza *et al.*[90] compared the rate of adaptive evolution (to glucose-limited media) in populations that were, or were not, recurrently recombined with migrant, novel genomes from an unrelated population that was not subject to the same selection regime. The experiment tested for an advantage of receiving (by migration and recombination) novel, unselected variation that might fortuitously be favoured in the new environment. No net improvement in adaptation to limiting glucose was detected in the recombining populations. However, there was evidence that migrant genes accumulated in the gene pool of the recombining lines due to selection, but not those that contributed to adaptation to the low glucose environment — that is, these genes influenced other fitness components.

Yeast have been used frequently to test the advantage of recombination in microorganisms. Birdsell and Wills[91] and Greig *et al.*[92] showed that when pairs of *Saccharomyces cerevisiae* strains were recombined, at least one of the many recombinant progeny lineages had a competitive advantage over the parental clones that produced them. These experiments show that recombination can produce a competitively superior genotype by recombining the parental genomes, but not that recombination, on balance, has a net advantage.

Zeyl and Bell[93] carried out a more crucial experiment by testing whether *S. cerevisiae* populations that experience recurrent recombination (sporulation) had an adaptive advantage compared with strictly clonal populations. Replicate populations with and without periodic sporulation were exposed to a new environment (galactose as a carbon source instead of glucose) or allowed to evolve for the same period of time in the ancestral environment (glucose carbon source). They found that recombination had no effect on the rate of adaptation to the new environment, but that populations with recombination evolved higher fitness than clonal populations when kept on the ancestral environment. The authors concluded that recombination speeded the elimination of harmful mutations but not the accumulation of beneficial mutations. However, if recombination speeded the elimination of harmful mutations, then recombining populations should have had elevated fitness in both the novel and original environments, and this was not observed. This inconsistency might be an artefact due to the extra selection on the recombining populations that were recurrently selected for growth on a novel (pre-sporulation) medium.

The most recent experiments that tested for accelerated progressive evolution with recombination used a *D. melanogaster* model system[78]. In these experiments, genome-wide synthetic chromosomes were constructed that were either recombining (neo-X) or non-recombining (neo-Y). New beneficial mutations were introduced into each of 34 replicated experiments and the fate of the beneficial mutations was traced with recombination present (17 neo-X treatments) and absent (17 neo-Y treatments). As predicted by theory, recombination sometimes helped and sometimes hurt the accumulation of the favoured mutation in individual experiments, but on average a strong advantage of recombination was observed. These experiments also showed that the variation in fitness among genetic backgrounds was substantial. This high-standing genetic variance in fitness would be expected to cause both faster progressive and slower retrogressive evolution in recombining populations (BOX 3).

GENE CONVERSION
The non-reciprocal transfer of genetic information between homologous genes (as a consequence of mismatch repair after heteroduplex formation).

Synthesis

We have made considerable experimental progress in showing the adaptive advantages of recombination. Studies of mutation rate show that it is large enough, at least among metazoans with long generation times, to create a debilitating mutational load in the absence of recombination. Experimental elucidation of the specific mechanisms that produce a load-reducing advantage of recombination (synergism among mutations, positive assortative mating for fitness or sexual selection) is still incomplete. Nonetheless, the hypotheses based on negative epistasis among functionally unrelated mutations and sexual selection are not supported by the available data. A significant challenge for future experiments will be to determine the mechanism(s) that reduce the mutational load of recombining species with high genome-wide mutation rates — for example, humans.

Experiments that measure the recovery of fitness (in asexual populations that had been repeatedly bottlenecked) show a large capacity for new beneficial and/or compensatory mutations to ameliorate the harm that is produced by the accumulation of harmful mutations. This finding indicates that beneficial, compensatory and/or reverse mutations might substantially reduce the mutational load, due to fixed deleterious mutations, of asexual species. This high potential for compensatory adaptation also might slow the rate at which the stochastic accumulation of deleterious mutations (retrogressive evolution) erodes the fitness of asexual lineages, especially when one large-effect advantageous mutation can mitigate the effects of multiple small-effect mutations.

Experiments with model systems that range from viruses to flies, on balance, confirm the theoretical prediction that recombination reduces background trapping and, thereby, both decreases the rate of accumulation of harmful mutations and increases the rate of accumulation of beneficial mutations. The magnitude of these advantages continually accrues over time. There is also limited support for the hypothesis that recombination reduces interference between beneficial mutations that are simultaneously segregating in a population. Future experiments need to address the relative importance of recombination in speeding progressive evolution versus retarding retrogressive evolution.

Last, recent experiments concerning directed evolution through exon shuffling indicate a potential advantage to recombination at the level of individual genes[79]. Most eukaryotic genes are segmented into coding exons that are separated by non-coding introns. Because introns tend to be much larger than exons, most intragenic crossovers occur within introns. This pattern of intragenic recombination shuffles intact exons from different homologous genes, or among members of the same gene family through GENE CONVERSION, creating new exon combinations that would require many mutational steps in a non-recombining gene. The relative importance of this process remains to be shown experimentally, but it might substantially extend the adaptive potential of recombining genes.

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