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Why do bacteria engage in homologous recombination?

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Microbiologists have long recognized that the uptake and incorporation of homologous DNA from outside the cell is a common feature of bacteria, with important implications for their evolution. However, the exact reasons why bacteria engage in homologous recombination remain elusive. This Opinion article aims to reinvigorate the debate by examining the costs and benefits that homologous recombination could engender in natural populations of bacteria. It specifically focuses on the hypothesis that homologous recombination is selectively maintained because the genetic variation it generates improves the response of bacterial populations to natural selection, analogous to sex in eukaryotes.

What is homologous recombination?

Recombination in bacteria is defined here as the incorporation of DNA from a donor cell into the genome of a recipient cell. In contrast to recombination involving meiosis and fertilization in eukaryotic lineages, DNA transfer in bacteria is unidirectional and always independent of reproduction (cell division). Bacterial recombination is often classified according to the mechanism by which foreign DNA is introduced in the cell: transduction (see Glossary) by bacteriophage, conjugation by plasmids or transformation by uptake of free DNA from the environment. An alternative classification focuses on the type of DNA being transferred: either a stretch of non-homologous or a stretch of homologous donor DNA. A novel gene can be transferred from non-homologous donor DNA, whereas for homologous donor DNA, the new variation is limited to a new (or identical) allele (Figure 1). Non-homologous recombination is often referred to as lateral gene transfer (LGT). Although the distinction between homologous recombination and LGT is often not made explicitly in discussions of bacterial recombination, the two processes can potentially engender very different adaptive benefits and it is necessary to distinguish clearly between them (Box 1).

This article explores the hypothesis that homologous recombination in bacteria (and archaea) is equivalent to sex in eukaryotes. By shuffling around alleles, homologous recombination results in an increase in genetic variation in the population, thereby potentially improving the response of the population to natural selection [1–4]. The first part of the article will review some of the most important models that have been proposed to explain the evolutionary benefits of sex and how they could apply to homologous recombination in bacteria. The second part of the article reviews the costs associated with DNA uptake and homologous recombination.

Explanations for sex

The Fisher-Muller model: climbing Mount Fitness

A powerful metaphor in evolutionary biology is that of the adaptive (or fitness) landscape [5]. This is a three-dimensional or two-dimensional plot of all possible genotypes in a given environment, with the fitness of each

Glossary

Allele: a particular version of a gene present at a locus.
Bottleneck: a reduction in population size, resulting in a decrease in genetic diversity.
Competence: a physiological state in which cells are able to take up exogenous DNA.
Conjugation: a process whereby DNA is transferred between cells that are in physical contact. The transferred DNA is typically in the form of a circular plasmid, which usually carries the genes responsible for the contact and transfer.
Diversifying selection: selection for many functional alleles at a locus. This process can be recognized by an excess of nonsynonymous nucleotide changes relative to synonymous nucleotide changes.
Effective population size: the number of cells contributing to future generations of the population; often many orders of magnitude smaller than the total (census) population size.
Lateral gene transfer (LGT): defined here as the incorporation of a non-homologous gene. Also referred to as horizontal gene transfer or illegitimate recombination.
Linkage disequilibrium: the non-random association between mutations that arises in clonally reproducing populations. Recombination shuffles mutations around to form novel combinations of existing mutations. This can decrease linkage disequilibrium up to the point where the presence of a particular mutation is no longer indicative of the presence of other mutations. This state is termed linkage equilibrium.
Recombination load: the (negative) difference in fitness between offspring produced by recombination and clonally produced offspring.
Sex: often more narrowly applied to meiosis and fertilization in eukaryotes, where both random assortment of chromosomes and recombination (crossing-over) generate variation. In this paper, the term 'sex' is also applied to any process that generates genetic variation by homologous recombination in bacteria.
Sign epistasis: interactions between mutations so that mutation A can confer a fitness advantage in genetic background X but a fitness disadvantage in genetic background Y. Sign (or physiological) epistasis should not be confused with statistical (or population) epistasis, which describes how mutations present in different individuals in a population interact.
Transduction: the transfer of donor cell DNA into the recipient cell by bacteriophage followed by recombination.
Transformation: the uptake of free DNA from the environment followed by recombination. Transformation is sometimes referred to as natural transformation to distinguish it from routinely performed artificial transformations in the lab.

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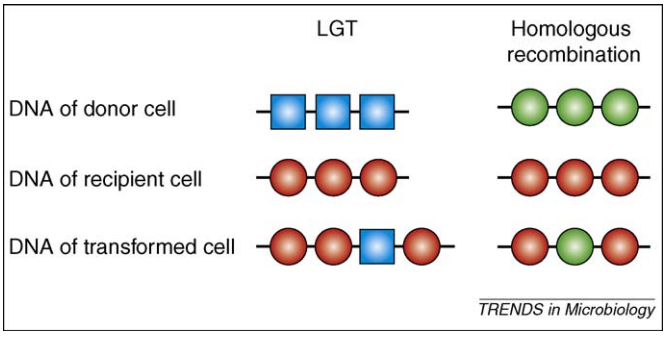

Figure 1. The difference between LGT (non-homologous recombination) and homologous recombination. Genes are depicted as coloured beads, with different genes differing both in colour and in shape and different alleles differing in colour but not in shape.

genotype represented by the height of the landscape. In the simplest scenario, this landscape has a single fitness peak. When bacteria find themselves in a new environment, they will most likely not inhabit this peak and new beneficial mutations will be needed to climb it. However, it is unlikely that these novel beneficial mutations will arise in the same individual. Instead, different beneficial mutations will crop up in different individuals (i.e. they will be in linkage disequilibrium). The subsequent competition between different beneficial mutations slows down the emergence of a single best-adapted clone, a process known as clonal interference.

Clonal interference depends crucially on population size. When populations are very small, there is little chance that a novel beneficial mutation will arise before a previous mutation has been fixed in the population. In populations that are very large, however, chances are that multiple beneficial mutations will arise in single individuals. Only in populations between these two extremes, arguably the most realistic populations, can clonal interference slow down adaptation. The Fisher-Muller model posits that homologous recombination brings competing beneficial mutations together into one genome, eliminat-

Box 1. Non-homologous recombination (LGT) versus homologous recombination

Bacterial genomes are commonly considered to be composed of a core genome, consisting of genes essential to survival that are shared by all related strains and species, and an accessory genome, consisting of non-essential genes that might or might not be present within a given strain. LGT transfers genes that make up the accessory genome, whereas homologous recombination affects both the accessory genome and the core genome. A recent analysis by Narra and Ochman using a variety of species revealed no clear relationship between the extent of homologous recombination and LGT [39], indicating that these two processes might be maintained by different selective pressures.

Knowledge about LGT has come primarily from genomic data, and so our view is inherently biased to occasions in which LGT has proven to be beneficial (or at least neutral) [40,41]. However, it is much more likely that the random incorporation of a gene will decrease, rather than increase, the fitness of the recipient cell. It has been shown that the acquisition of new genes involved in metabolism is more likely to be successful if genes encoding physiologically coupled enzymes are already present in the genome [42]. The incorporation of foreign genes as a mechanism to acquire novel phenotypes thus might or might not result in increased fitness of the recipient cell (Table I).

Although many bacterial genomes have been profoundly shaped by LGT [43], it is important to realize that the pervasiveness of LGT in itself cannot be taken as evidence that it has evolved as an adaptation to acquire new genetic information [40]. LGT often results from the transfer of mobile genetic elements. Only when the cell has evolved mechanisms to mediate conjugation and transduction can LGT be classified as an adaptation (regardless of whether certain individual recombination events are adaptive) [34] (Table I). This could be true for conjugative plasmids but is less likely for selfishly replicating bacteriophages. It is possible that LGT via transformation occurs as a by-product of incorporating homologous DNA for repair or sex, with bacteria encountering more diverse sources of DNA being more prone to incorporate non-homologous DNA by accident (Table I).

An alternative explanation for the incorporation of foreign DNA in the genome is that it is a by-product of the uptake of DNA to use nucleotides as precursors for host DNA or as a source of energy [34]. Because all sequences consist of purines and pyrimidines, this hypothesis is valid for both types of recombination (Table I). Indeed, *Escherichia coli* can grow on both homologous and non-homologous double-stranded DNA as the sole source of carbon and energy [44]. The specificity of DNA uptake, however, is known to be restricted in some species in which genus-specific sequence repeats are necessary for successful DNA uptake (e.g. *Neisseria gonorrhoeae* and *Haemophilus influenzae*) [45]. Specificity of DNA uptake presents a major problem for the generality of the DNA metabolism hypothesis: why would these bacteria not feed on non-homologous DNA? A different argument brought forward against this hypothesis is that some species (e.g. *Bacillus*) are also capable of efficiently digesting DNA extracellularly and taking up the individual nucleotides, precluding the need for the uptake of single-stranded DNA [46], although it is not known how the cost of taking up single nucleotides compares with the cost of taking up nucleotide strands.

Homologous recombination is mediated by *rec* genes, the primary function of which is recombinational repair of DNA damage [40]. Note that DNA damage refers to double-strand breaks and other structural alterations that can be recognized by repair enzymes and not to deleterious mutations that are invisible to this machinery [30]. It has been proposed that foreign DNA is taken up to serve as a template to repair damaged homologous stretches of DNA [30]. Normal aerobic metabolism, the production of endogenous hydrogen peroxide (to inhibit competing species) and exposure to leukocyte oxidative bursts in host immune responses can lead to extensive DNA damage, and the high rate of homologous recombination in several pathogens and commensals has been proposed to serve to repair this damage [30]. Of all explanations for bacterial recombination, the DNA repair hypothesis has perhaps received most experimental attention, but there is considerable disagreement over the interpretation of results [30,40]. Higher numbers of transformed cells are observed in *Bacillus* with increasing levels of UV irradiation, but this seems to be primarily caused by a general upregulation of recombination processes and not increased survival of recombinants because of DNA repair [30,47].

Table I. The most commonly invoked explanations for the incorporation of foreign DNA in bacteria[a]

Non-homologous recombination (LGT)		Homologous recombination	
Indirect benefit:	acquiring novel genetic diversity	Indirect benefit:	creating novel combinations of alleles (sex)
By-product of:	infectious gene transfer	By-product of:	infectious gene transfer
By-product of:	metabolizing DNA	By-product of:	metabolizing DNA
By-product of:	homologous recombination for sex or repair	Direct benefit:	DNA repair

[a]Hypotheses are classified as 'direct benefit', whereby recombination results in a fitness increase of every recipient cell; 'indirect benefit', whereby recombination results in increased fitness of some recipient cells but not others (and so only confers a benefit on the level of the population); or 'by-product of', whereby recombination is a side-effect of another biological process. It should be noted that the different hypotheses are not mutually exclusive and could act alongside each other [30]. (Note that infectious gene transfer does not necessarily confer a direct benefit to the host cell but does confer a direct benefit to the mobile genetic element involved.).

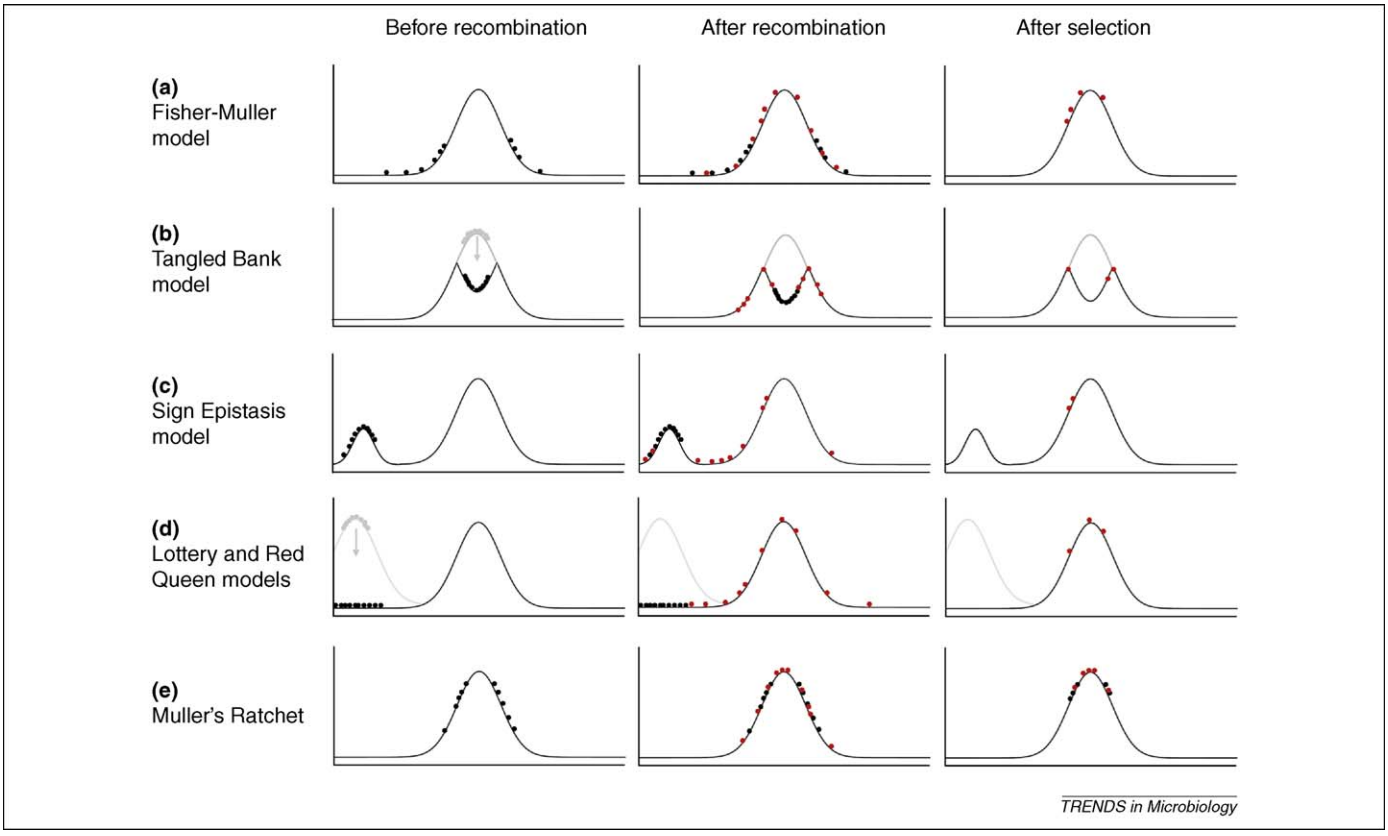

Figure 2. Two-dimensional representations of fitness landscapes. The *x*-axis represents a continuum of different genotypes, and the *y*-axis represents fitness. The first panel shows a population of different genotypes (black dots) in a fitness landscape. The second panel shows these genotypes in addition to genotypes produced by homologous recombination (red dots). Recombination increases the spread of genotypes over the landscape (i.e. increases fitness variance). The third panel shows the genotypes that remain after selection has removed genotypes occupying the lower half of the landscape. **(a)** In the Fisher-Muller model, recombination enables populations to climb a fitness peak faster than they can through mutation. **(b)** In the tangled bank model, recombination helps populations escape from a fitness peak depressed by intense resource competition (the past fitness peak is shown in grey; genotypes that used to occupy its top drop down to the present landscape). **(c)** In the sign epistasis model, recombination enables populations stuck on low fitness peaks to reach the highest fitness peak. **(d)** Although different forces underlie changes in the fitness landscape in the Lottery and Red Queen models, both assume that the increased spread of genotypes over the fitness landscape through recombination enables populations to reach novel peaks more rapidly. **(e)** Muller's Ratchet causes populations to slide from their adaptive peaks. Recombination enables the reconstruction of genotypes free from deleterious mutations.

ing clonal interference and speeding up adaptation (Figure 2a). The Fisher-Muller model is one of the best established explanations for sex and, therefore, has attracted most experimental attention from microbiologists [4,6,7] (Box 2).

The tangled bank model: a crowded summit

Once a population has climbed a fitness peak, there is a chance that it will fall victim to its own success: when the population gradually depletes resources and competition intensifies, growth rate slows down. In the fitness landscape analogy, the top of the fitness peak will gradually become depressed, making surrounding regions more favourable (Figure 2b). Thus, fitness landscapes are not rigid but change shape depending on the position and density of occupying populations (much like a bouncy castle changes shape under the weight of a group of children). The tangled bank model posits that recombination

Box 2. Experimental evidence that homologous recombination can reduce clonal interference in bacteria

An experimental evolution experiment by Cooper [7] elegantly provides evidence that bacterial recombination can act as an equivalent to eukaryotic sex by reducing clonal interference. Eight replicates of four *E. coli* lines, genetically engineered at one or two loci, were allowed to evolve in a novel laboratory environment. Knocking out one locus resulted in the mutant being unable to engage in homologous recombination via the F-plasmid (*rec*$^-$); knocking out the other locus resulted in mutation rates elevated 30-fold compared with the wild type. Rates of adaptation did not differ significantly between the *rec*$^+$ and *rec*$^-$ lines with wild-type mutation rate because beneficial mutations did not arise frequently enough to result in clonal interference. In the two high-mutation-rate lines, however, adaptation proceeded more rapidly in *rec*$^+$ than in *rec*$^-$ lines because multiple beneficial mutations were competing in the *rec*$^-$ clones but could act together after recombination in the *rec*$^+$ clones. Clonal interference could be verified by the identification of a single beneficial mutation in a candidate locus that was followed by sequencing clones in different lines at different time points in the evolution experiment. This particular mutation was found to increase in frequency at a slower rate in the *rec*$^-$ lines and conferred a smaller adaptive benefit because it had to compete with other beneficial mutations in the population.

Although the large population sizes, the short generation times and the availability of molecular genetic tools facilitate experimental tests of the various hypotheses for the benefit of recombination in bacteria, compared to most eukaryotes, several difficulties exist as well. Conditions that are relevant for bacteria in nature are hard to mimic in the laboratory (provided they have been identified in the first place), making it possible that experiments are performed under suboptimal or even irrelevant conditions. In addition, the complex molecular machinery involved in recombination could be pleiotropically linked to other important cellular functions that are under selection during laboratory evolution.

helps the population to diversify to escape competition and enhance overall population growth [8,9]. To the author's knowledge, the tangled bank model has not received any attention in the context of bacterial recombination. However, it could potentially apply to bacteria that grow to high local population densities and cannot escape competition by dispersal.

The sign epistasis model: through the valley of death

The previous models focus on smooth fitness landscapes containing a single peak. This is the case when mutations affect fitness independently from all other mutations (some mutations take every genotype higher up, whereas others will take every genotype lower down). However, mutations tend to influence each other's effect on fitness: mutations that are beneficial in one genetic background are not necessarily beneficial in another background. This phenomenon, known as sign epistasis, results in fitness landscapes becoming rugged, with many small and large peaks, ridges and valleys [4,10,11]. Sign epistasis seems to be the rule rather than the exception in most types of organisms, including bacteria [5,11]. In a smooth landscape, populations will always arrive at the highest peak – either slowly, via mutation, or faster, aided by recombination. Populations in a rugged landscape, however, will often climb suboptimal peaks and become stuck there because the only mutations that are accessible to them will bring them down into fitness valleys [11,12]. Recombination could potentially help populations traversing fitness valleys to reach the highest peak in the landscape (Figure 2c).

The lottery model: a leap in the dark

The models discussed above consider adaptation in static environments. However, it is obvious that populations will also experience environmental change, either because local conditions change over time or because cells are dispersed to new localities. (Note that when selection pressures do not change, recombination would remove populations from the highest fitness peak they had climbed and could not be beneficial.) When fitness peaks frequently change position in the fitness landscape, recombination could serve to produce a variety of different offspring in the hope that one of them will occupy a spot on the landscape where the next fitness peak will appear (Figure 2d). This scenario is known as the lottery model [13], after the analogy that it is better to hedge one's bets and buy different lottery tickets than to buy the same number of identical tickets.

Bacteria are likely to face new selective challenges after a change of the local environment or dispersal to new environments, but the degree to which this occurs must differ between different microbial communities. Imagine, for instance, extremely heterogeneous soil environments, in which any single colony borders only a limited number of resource patches (compared to the total number present in the entire plot of land). By contrast, cells in aquatic environments are expected to encounter many more combinations of resources owing to frequent dispersal, resulting in varying selection pressures. Cells in aqueous environments are also expected to encounter other genotypes more frequently, which is essential for homologous recombination to generate variation in a population. Although very few data are available, aquatic bacteria tend to have elevated homologous recombination rates compared with terrestrial bacteria [14]. It must be noted, however, that an increased degree of population mixing alone could result in a stronger signature of homologous recombination, without any particular benefit of novel allele combinations.

The Red Queen model: mountains that move

In lottery models, the environment is usually assumed to be abiotic, merely consisting of different combinations of resources (see, for instance, Ref. [9]). However, most bacteria find themselves in immensely complex microbial communities, and changes in the biotic environment are likely to be very important as well. Selection exerted by competing genotypes and species, predators (e.g. protozoans) and parasites (bacteriophages) is likely to be not only very strong but also continuous because a new adaptation by one species (e.g. infectivity) will select for a counter-adaptation in the other species (e.g. resistance), and so forth. This dynamic process of antagonistic co-evolution is one of the most popular explanations for sex in eukaryotes [3,15,16]. The name of the model is taken from Lewis Carroll's book *Through the Looking-Glass*, where the Red Queen tells Alice: 'It takes all the running you can do, to keep in the same place'.

Lytic bacteriophages exert a strong selection pressure on bacteria to evolve to become resistant [17]. It seems reasonable to assume that host-to-host transmission of lytic bacteriophages is achieved much more easily in unstructured environments [18]. Accordingly, aquatic environments seem to have a higher number of bacteriophages (relative to the number of bacteria) than terrestrial environments do [18]. This leads to the intriguing hypothesis that elevated levels of co-evolution between bacteria and phages could select for the high homologous recombination rates observed in bacteria inhabiting aqueous environments [14].

Researchers have focused almost entirely on the importance of sex for hosts because the short generation times, high mutation rates and large population sizes of parasites have usually been assumed to provide beneficial mutations at a sufficient rate. However, it could be envisaged that homologous recombination could increase the rate of adaptation of bacterial parasites as well. Surface-expressed proteins in pathogenic bacteria elicit strong selection for recognition by the immune system of eukaryote hosts and, as a consequence, have diversified to escape this recognition. Whereas mutation can only alter alleles in small steps, homologous recombination enables cells to obtain highly divergent alleles in a single evolutionary event, thus potentially dramatically speeding up adaptation (Box 3). Moreover, novel alleles could be created by combining parts of both alleles [19]. In agreement, many examples exist of virulence genes for which evidence exists of both diversifying selection and homologous recombination (see, for instance, Ref. [20]) and pathogenic *Escherichia coli* lineages have been shown to engage more frequently in homologous recombination than non-pathogenic lineages [21].

Box 3. Creating new alleles through homologous recombination

The efficiency of homologous recombination declines with sequence divergence between donor and recipient DNA in a log-linear manner because of a variety of molecular constraints [48]. It is important, however, to realize that homologous recombination can extend beyond homologous sequence. Proteins consist of a variety of conserved motifs for structural stability, as well as more variable sequence motifs involved in substrate specificity and activity. Even highly divergent stretches of sequence can be incorporated into a genome by homologous recombination as long as they are flanked by conserved sequences. This principle is employed to generate novel gene products in laboratory-directed protein evolution, where it is known as DNA shuffling [49]. In this procedure, homologous but divergent sequences from different species are fragmented, followed by reassembly of the fragments in a self-priming polymerase reaction. The resulting chimaeric sequences consist of conserved regions where homologous recombination has taken place interspersed with variable regions. Homology-dependent recombination can thus result in great leaps in sequence space and can dramatically increase responsiveness to selection relative to the accumulation of point mutations [49].

Muller's Ratchet: sliding down the mountain

No matter whether a population is climbing a single fitness peak or aiming for a new peak, deleterious mutations will occur along with beneficial ones (in fact, most mutations are deleterious; random genetic change is more likely to do harm than good). Although the most severe deleterious mutations will be removed directly because they render their bearers non-viable, less deleterious mutations will inevitably accumulate even in the fittest genomes. This process is called Muller's Ratchet. Without selection, every generation will be a random subsample of the previous generation. When the size of this subsample is small, sampling error will become important (a process known as genetic drift). Populations that go through repeated genetic bottlenecks thus run the risk of losing individuals that are free of deleterious mutations and will slowly but surely slide down the fitness peak. Homologous recombination can counteract this process by reconstructing mutation-free individuals (Figure 2e).

Muller's Ratchet has been shown to cause a decrease in bacterial fitness when laboratory populations are forced to pass through a single-cell bottleneck regularly for a prolonged time [22]. Although the effective population size of bacteria is usually assumed to be enormous and genetic drift seems unlikely to be generally strong, genetic bottlenecks could still be important in some cases; for example, in endosymbionts in which limited numbers of cells are transmitted from host to host [23]. The subdivision of the global population into local populations will also result in a greater influence of drift and a greater potential benefit of recombination [24]. Spatial population structure can be particularly strong in extremophiles inhabiting isolated habitats such as hot springs (see, for instance, Ref. [25]), but even populations of free-living bacteria inhabiting contiguous environments, without obvious barriers to gene flow, can be structured [26].

The costs of recombination in bacteria

One of the greatest problems evolutionary biologists have had in explaining the ubiquity of sex in eukaryotes is the high cost of sex, which decreases its potential evolutionary benefit [2]. A main cost of sex is the recombination load, or the loss of population fitness caused by the breakup of beneficial allele combinations by recombination [2]. Because the allele combinations of individuals alive today must have survived many rounds of natural selection, they will, on average, be fitter than any random combination produced by recombination. The decrease in fitness caused by recombination load in the short run must thus be outweighed by an increase in fitness by more efficient natural selection in the long run for recombination to be a successful strategy.

The importance of recombination load depends on the shape of the fitness landscape and the position of populations in it. When genotypes find themselves high on an adaptive peak, novel genotypes produced by recombination are more likely to end up away from the top than closer to it, so recombination load is high. The overall fitness decrease of the population in the short term, however, can still be outweighed in the longer term when recombination is better able to produce even a single, much fitter genotype than mutation. When genotypes find themselves in a fitness valley, recombination is unlikely to decrease fitness even further and recombination can increase overall population fitness in the short term, as well as in the long term.

A major cost of sex in many eukaryote species is the cost of males. The *per capita* growth rate of asexual populations is twice that of sexual populations because every single individual, not pairs of individuals, produces offspring. This twofold cost of sex is absent in bacteria in which all cells in the population are of the same type. However, bacterial sex is still associated with considerable costs. Bacteria need to be in a particular physiological state, termed competence, to be able to take up foreign DNA and engage in recombination. Free DNA taken up by cells via transformation can originate from cells in the population that have lysed. For instance, in *Streptococcus pneumoniae*, competent cells lyse non-competent cells through cell-to-cell contact, releasing DNA that is then available for uptake [27]. A wide variety of species are also known to actively secrete DNA into the environment that is then available for uptake by other cells [28,29]. Non-recombining mutants that do not carry costs of DNA uptake, DNA secretion or active cell lysis (the cost of cell death, as well as the cost of killing) are expected to have a growth advantage in the absence of any fitness benefits associated with recombination. Only when the long-term risk of extinction is higher in clonal populations can recombining populations thus persist.

Active secretion of DNA could be used as an argument against the DNA metabolism hypothesis [30] and interpreted as an adaptation facilitating the repair and sex hypotheses. An alternative evolutionary explanation for the secretion of DNA that has been brought forward recently, and is related to infective gene transfer by bacteriophages and plasmids (Box 1), is that genes promoting DNA secretion can selfishly spread through the population by transformation (along with other genes; there is no evidence that specific portions of the genome are secreted) [29]. However, this hypothesis lacks empirical support,

ignores the potential benefits that DNA secretion and recombination could confer to populations and does not explain why cells would actively take up DNA from the environment in the first place.

It has been observed in many facultatively sexual eukaryotes that sex takes place primarily when conditions are suboptimal. One hypothesis that explains this phenomenon is that the costs associated with sex might be less pronounced under harsh conditions because competition for resources is minimal [8]. A wide variety of bacteria and archaea can develop competence [28], but the efficiency of transformation is often highly sensitive to physiological, nutritional and temperature conditions (see, for instance, Ref. [31]). Competence is often, but not universally, induced by stress. In *Vibrio cholerae*, for example, competence is induced by nutrient limitation and increased cell density but also by growth on chitin [32]. Whatever the exact triggers for DNA uptake and recombination, tight regulation of competence could potentially greatly lower the cost of recombination and, therefore, increase its potential benefit.

A study by Fall *et al.* [33] on *Ralstonia solanacearum* provides experimental evidence that transformation efficiencies can differ substantially between loci. The observed recombination 'hotspots' correlated with the presence of short signature sequences resembling so-called Chi sequences known to promote DNA repair by homologous recombination in *E. coli*. This finding indicates that it could theoretically be possible that homologous recombination rate is increased around loci prone to be involved in adaptation, such as surface antigens, thereby increasing its effectiveness and decreasing its cost.

Concluding remarks

It is evident that some benefit must be associated with homologous recombination, whether directly, indirectly or through a linked process of which the incorporation of foreign DNA in the genome is a by-product (Table 1). The simplest demonstration for the existence of such a benefit is the fact that DNA uptake and subsequent recombination would otherwise not be found: any non-recombining mutant would outcompete recombining cells because it would not bear the costs associated with recombination. Furthermore, a non-recombining mutant would convert recombining cells to non-recombining cells by providing them with DNA with recombination-deficient loci [34].

This article has made a case for the possibility that homologous recombination is selectively favoured in bacteria because it facilitates natural selection. The various hypotheses brought forward to explain the benefits of sex in eukaryotes are equally applicable to bacteria, probably even more so because the cost of bacterial recombination could be lower. Although the different hypotheses for why sex might be beneficial all have intuitive appeal and varying degrees of empirical and theoretical support, none of them is generally regarded to be the ultimate explanation for sex. Instead, it is most likely that multiple mechanisms act alongside each other, possibly synergistically [35].

It is clear that the adaptive landscapes bacteria inhabit are rugged and that they change in shape over time because of competition, dispersal, fluctuations in the environment and coevolution with other species. Recent genomic studies indicate that a considerable portion of genes in bacteria can be under positive selection [36,37]. The more genes that are under selection, the more important clonal interference will be as an impediment to adaptive evolution. Importantly, deleterious mutations will also interfere with beneficial mutations. Theoretical work has shown that recombination can be effective in liberating beneficial mutations from bad genetic backgrounds under a wide range of conditions [38].

A greater appreciation of the fascinating diversity of bacterial life styles by evolutionary biologists, as well as a better understanding of evolutionary theory by microbiologists, will be essential to better understand the advantages associated with homologous recombination in bacteria. This, in turn, could greatly improve our understanding of bacterial evolution, speciation, genome architecture, ecosystem functioning and disease prevention. Box 4 lists outstanding questions in several areas of research. Elucidating the function of bacterial recombination will undoubtedly prove to be one of the most exciting topics in microbiology and evolutionary biology in years to come.

Box 4. Outstanding questions

- Intraspecific variation in transformability has been demonstrated in several species (e.g. *Pseudomonas stutzeri* [50]). Under what environmental conditions could strains with different recombinogenic potential outcompete each other? This could be tested using microcosm experiments.
- Genomic surveys can indicate the prevalence of homologous recombination in different chromosomal regions, as well as the presence or absence of sequence repeats that could represent potential DNA uptake or Chi-like marker sequences [33,51]. Does the density of Chi-like markers correlate with elevated homologous recombination rates? The comparison of genomic sequences from multiple strains belonging to the same species might help answer this question.
- A recent study demonstrated that homologous recombination rate can vary widely within phyla but is generally similar between species belonging to the same genus [14]. Is this the result of phylogenetic constraints or shared adaptive strategies?
- It is largely unknown how micro-scale environmental heterogeneity in space and time correlates with the genetic diversity of bacterial populations in nature [52]. Do local bacterial populations evolve to occupy new niches that become available to them or will niches mostly be filled by immigrant clones?
- The tangled bank and lottery models have largely fallen out of favour as explanations for the occurrence of sex in eukaryotes. However, could these models be applicable to the parameter space inhabited by bacterial populations?

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References

1 Agrawal, A.F. (2006) Evolution of sex: why do organisms shuffle their genotypes? *Curr. Biol.* 16, R696–R704
2 Otto, S.P. and Lenormand, T. (2002) Resolving the paradox of sex and recombination. *Nat. Rev. Genet.* 3, 252–261
3 Otto, S.P. and Gerstein, A.C. (2006) Why have sex? The population genetics of sex and recombination. *Biochem. Soc. Trans.* 34, 519–522
4 de Visser, J.A. and Elena, S.F. (2007) The evolution of sex: empirical insights into the roles of epistasis and drift. *Nat. Rev. Genet.* 8, 139–149

5 Colegrave, N. and Buckling, A. (2005) Microbial experiments on adaptive landscapes. *Bioessays* 27, 1167–1173
6 Baltrus, D.A. *et al.* (2008) Natural transformation increases the rate of adaptation in the human pathogen *Helicobacter pylori*. *Evolution* 62, 39–49
7 Cooper, T.F. (2007) Recombination speeds adaptation by reducing competition between beneficial mutations in populations of *Escherichia coli*. *PLoS Biol.* 5, e225
8 Bell, G. (1982) *The Masterpiece of Nature, The Evolution and Genetics of Sexuality.* Croom Helm
9 Scheu, S. and Drossel, B. (2007) Sexual reproduction prevails in a world of structured resources in short supply. *Proc. Biol. Sci.* 274, 1225–1231
10 Weinreich, D.M. *et al.* (2006) Darwinian evolution can follow only very few mutational paths to fitter proteins. *Science* 312, 111–114
11 Weinreich, D.M. *et al.* (2005) Perspective: sign epistasis and genetic constraint on evolutionary trajectories. *Evolution* 59, 1165–1174
12 Clune, J. *et al.* (2008) Natural selection fails to optimize mutation rates for long-term adaptation on rugged fitness landscapes. *PLOS Comput. Biol.* 4, e1000187
13 Williams, G.C. (1975) *Sex and Evolution.* Princeton University Press
14 Vos, M. and Didelot, X. (2008) A comparison of homologous recombination rates in bacteria and archaea. *ISME J.* 3, 199–208
15 Hamilton, W.D. *et al.* (1990) Sexual reproduction as an adaptation to resist parasites (a review). *Proc. Natl. Acad. Sci. U. S. A.* 87, 3566–3573
16 Agrawal, A.F. (2006) Similarity selection and the evolution of sex: revisiting the red queen. *PLoS Biol.* 4, e265
17 Brockhurst, M.A. *et al.* (2007) Experimental coevolution with bacteria and phage: the *Pseudomonas fluorescens*-Phi2 model system. *Infect. Genet. Evol.* 7, 547–552
18 Weinbauer, M.G. (2004) Ecology of prokaryotic viruses. *FEMS Microbiol. Rev.* 28, 127–181
19 Riley, M.A. and Wertz, J.E. (2002) Bacteriocins: evolution, ecology, and application. *Annu. Rev. Microbiol.* 56, 117–137
20 Orsi, R.H. *et al.* (2008) Lineage specific recombination and positive selection in coding and intragenic regions contributed to evolution of the main *Listeria monocytogenes* virulence gene cluster. *Infect. Genet. Evol.* 8, 566–576
21 Wirth, T. *et al.* (2006) Sex and virulence in *Escherichia coli*: an evolutionary perspective. *Mol. Microbiol.* 60, 1136–1151
22 Andersson, D.I. and Hughes, D. (1996) Muller's ratchet decreases fitness of a DNA-based microbe. *Proc. Natl. Acad. Sci. U. S. A.* 93, 906–907
23 Herbeck, J.T. *et al.* (2003) A conservative test of genetic drift in the endosymbiotic bacterium *Buchnera*: slightly deleterious mutations in the chaperonin groEL. *Genetics* 165, 1651–1660
24 Martin, G. *et al.* (2006) Selection for recombination in structured populations. *Genetics* 172, 593–609
25 Whitaker, R.J. *et al.* (2003) Geographic barriers isolate endemic populations of hyperthermophilic archaea. *Science* 301, 976–978
26 Vos, M. and Velicer, G.J. (2008) Isolation by distance in the spore-forming soil bacterium *Myxococcus xanthus*. *Curr. Biol.* 18, 386–391
27 Steinmoen, H. *et al.* (2003) Competence-induced cells of *Streptococcus pneumoniae* lyse competence-deficient cells of the same strain during cocultivation. *J. Bacteriol.* 185, 7176–7183
28 De Vries, J. and Wackernagel, W. (2004) Microbial horizontal gene transfer and the DNA release from transgenic crop plants. *Plant Soil* 266, 91–104
29 Draghi, J.A. and Turner, P.E. (2006) DNA secretion and gene-level selection in bacteria. *Microbiology* 152, 2683–2688
30 Michod, R.E. *et al.* (2008) Adaptive value of sex in microbial pathogens. *Infect. Genet. Evol.* 8, 267–285
31 Baur, B. *et al.* (1996) Genetic transformation in freshwater: *Escherichia coli* is able to develop natural competence. *Appl. Environ. Microbiol.* 62, 3673–3678
32 Meibom, K.L. *et al.* (2005) Chitin induces natural competence in *Vibrio cholerae*. *Science* 310, 1824–1827
33 Fall, S. *et al.* (2007) Horizontal gene transfer regulation in bacteria as a "spandrel" of DNA repair mechanisms. *PLoS One* 2, e1055
34 Redfield, R.J. (1993) Genes for breakfast: the have-your-cake-and-eat-it-too of bacterial transformation. *J. Hered.* 84, 400–404
35 West, S.A. *et al.* (1999) A pluralist approach to sex and recombination. *J. Evol. Biol.* 12, 1003–1012
36 Charlesworth, J. and Eyre-Walker, A. (2006) The rate of adaptive evolution in enteric bacteria. *Mol. Biol. Evol.* 23, 1348–1356
37 Lefebure, T. and Stanhope, M.J. (2007) Evolution of the core and pan-genome of *Streptococcus*: positive selection, recombination, and genome composition. *Genome Biol.* 8, R71
38 Keightley, P.D. and Otto, S.P. (2006) Interference among deleterious mutations favours sex and recombination in finite populations. *Nature* 443, 89–92
39 Narra, H.P. and Ochman, H. (2006) Of what use is sex to bacteria? *Curr. Biol.* 16, R705–R710
40 Redfield, R.J. (2001) Do bacteria have sex? *Nat. Rev. Genet.* 2, 634–639
41 Gogarten, J.P. and Townsend, J.P. (2005) Horizontal gene transfer, genome innovation and evolution. *Nat. Rev. Microbiol.* 3, 679–687
42 Pal, C. *et al.* (2005) Adaptive evolution of bacterial metabolic networks by horizontal gene transfer. *Nat. Genet.* 37, 1372–1375
43 Ochman, H. *et al.* (2000) Lateral gene transfer and the nature of bacterial innovation. *Nature* 405, 299–304
44 Finkel, S.E. and Kolter, R. (2001) DNA as a nutrient: novel role for bacterial competence gene homologs. *J. Bacteriol.* 183, 6288–6293
45 Hamilton, H.L. and Dillard, J.P. (2006) Natural transformation of *Neisseria gonorrhoeae*: from DNA donation to homologous recombination. *Mol. Microbiol.* 59, 376–385
46 Dubnau, D. (1999) DNA uptake in bacteria. *Annu. Rev. Microbiol.* 53, 217–244
47 Michod, R.E. and Wojciechowski, M.F. (1994) DNA repair and the evolution of transformation IV. DNA damage increases transformation. *J. Evol. Biol.* 7, 147–175
48 Thomas, C.M. and Nielsen, K.M. (2005) Mechanisms of, and barriers to, horizontal gene transfer between bacteria. *Nat. Rev. Microbiol.* 3, 711–721
49 Crameri, A. *et al.* (1998) DNA shuffling of a family of genes from diverse species accelerates directed evolution. *Nature* 391, 288–291
50 Sikorski, J. *et al.* (2002) Highly different levels of natural transformation are associated with genomic subgroups within a local population of *Pseudomonas stutzeri* from soil. *Appl. Environ. Microbiol.* 68, 865–873
51 Mau, B. *et al.* (2006) Genome-wide detection and analysis of homologous recombination among sequenced strains of *Escherichia coli*. *Genome Biol.* 7, R44
52 Hunt, D.E. *et al.* (2008) Resource partitioning and sympatric differentiation among closely related bacterioplankton. *Science* 320, 1081–1085

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