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Of What Use Is Sex to Bacteria?

Review

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Though bacteria are predominantly asexual, the genetic information in their genomes can be expanded and modified through mechanisms that introduce DNA from outside sources. Bacterial sex differs from that of eukaryotes in that it is unidirectional and does not involve gamete fusion or reproduction. The input of DNA during bacterial sex generates diversity in two ways — through the alteration of existing genes by recombination and through the introduction of novel sequences — and each of these processes has been shown to aid in the survival and diversification of lineages.

Free-living bacteria reproduce by binary fission, whereby a single cell replicates its genome and divides into two identical daughter cells. In this system, variation is introduced into a lineage largely by mutations and sometimes by the intragenomic duplication of the existing genetic information. When limited to these processes, the generation of variation at the DNA level and the origin and spread of novel traits at the phenotypic level can be relatively slow processes in asexually reproducing lineages. Given that most mutations of any consequence to the organism (i.e., non-neutral mutations) are harmful, asexual lineages also suffer from the accumulation of deleterious mutations. Because the original allele cannot be recovered, except by the very event of a back mutation, H.J. Muller likened this process to an irreversible ratchet (and asexual lineages are often said to suffer from 'Muller's ratchet'). Naturally, both sexual reproduction (in which alleles from different organisms are merged) as well as recombination bring together new combinations of alleles over loci in each generation and can help overcome some of the drawbacks associated with asexuality. On the other hand, sexual reproduction disrupts combinations of unlinked beneficial mutations and, in such cases, asexual reproduction, in producing uniform progeny, would be analogous to winning a lottery. The role of selection on evolution of bacterial lineages has been addressed experimentally: *Escherichia coli* grown under starvation conditions were able to survive for several years, and the strain that arose over the course of the experiment was able to replace the parental strain in mixed culture competitions [1,2]. Although this shows that certain clones can take over and become fixed in the population, such experiments may not mimic natural conditions in which parasites (viruses and phages in this case) adapt to the most common clone. Under such circumstances of frequency-dependent selection, sexual reproduction constantly re-assorts genotypes, keeping organisms one step ahead of their infectious agents (known as the Red Queen Hypothesis).

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Considering Sex in Bacteria

Despite their asexual mode of reproduction, bacteria have sex, or at least something that is referred to as sex and can be defined as the inheritance of DNA from any source aside from the parental cell. Unlike the sexual process occurring in most eukaryotes, the transfer of genetic material during bacterial sex is unidirectional and can occur by one of three mechanisms that differ with respect to the source of DNA and/or the types of partners involved. Although these processes — conjugation, transformation and transduction — were each characterized decades ago (see Thomas and Nielsen [3] for an excellent review), the availability of complete bacterial genome sequences has brought renewed interest in their contributions to the contents and organization of bacterial genomes, their consequences on attempts to reconstruct the phylogeny and relationships among bacteria, and their role in bacterial adaptation and the dissemination of disease determinants.

Mechanisms for DNA Transfer and Uptake in Bacteria

Conjugation typically transfers DNA from donor to recipient cells and can occur by: self-transmissible and mobilizable plasmids termed F (sex factor) plasmids; integrative conjugative elements, i.e., plasmids that integrate into chromosome forming high frequency recombinant (Hfr) strains; and conjugative transposons, which encode proteins for their excision and transposition into the recipient strains. Although conjugation usually involves exchange between homologous stretches of DNA, the occurrence of transposable elements within the transferred regions can result in heterologous exchange between the mating pair. Conjugation need not only occur between donor and recipient strains of the same species, because DNA transfer by this mechanism has even been reported between bacteria and yeast [4], between bacteria and plants [5], and between bacteria and mammalian cells [6].

The conjugational apparatus of bacteria is composed of a translocation channel, which spans the cell envelope, and either surface filaments in Gram-negative bacteria or surface adhesin proteins in Gram-positive bacteria [7]. In *Agrobacterium tumefaciens*, where conjugation has been intensely studied due to its application in plant genetic engineering, the mating pair proteins along with several coupling proteins, all present in the donor cell, mediate the transfer of DNA. In addition, several virulence genes (*vir* genes) along with mating proteins have recently been shown to enable T-DNA transfer into plants [8,9].

Transformation involves steps common to many bacteria: development of competence (i.e., an ability to take up DNA from the environment); binding of DNA to the surface; stable uptake; and integration into the chromosome via recombination with its homologous counterpart. Gram-negative bacteria vary

slightly in the process of DNA uptake due to the presence of an extra outer membrane and periplasmic space [10,11]. DNA transformation can potentially transmit gene-bearing fragments or circular plasmids between very distantly related species. Many bacterial genera, including *Campylobacter* [12], *Haemophilus* [13], *Helicobacter* [14], *Neisseria* [15,16] and *Pseudomonas* [17], are naturally transformable throughout their life cycle, whereas others, such as *Bacillus* and *Streptococcus,* are competent only during specific physiological states [18].

Efficient transformation of *N. gonorrhoeae* and *H. influenzae* requires the presence of species-specific sequences of ten (GCCGTCTGAA) and nine (AAGTGC GGT) nucleotides in their genomes, respectively [19,20]. However, transformation efficiency varies even in the presence of the specific recognition sequences. Recently, it was found that chitin (as present in the shells of crustacea, insect skeletons, fungal cell walls, and diatoms), cell density and nutrient stress were all capable of inducing competence in *Vibrio cholerae* [21]. In this system, the *V. cholerae* TfoX domains are induced by chitin, which in turn causes the expression of several other genes responsible for chitin degradation, chitin-induced competence and type IV secretion system (*pilA*, *pilB* and *pilQ*), processes required for transformation.

In addition to taking up DNA from the environment, some Gram-negative bacteria have membrane-based vesicles (transformasomes), which provide an alternative mode of gene transfer [22]. These small, spherical vesicles encapsulate periplasmic components, including periplasmic proteins, phospholipids, DNA and RNA [23], and are also known to contain several virulence factors, such as hemolysin, autolysin and Shiga toxin, implicating a role for these vesicles in virulence [24,25]. These bacterial vesicles can deliver plasmid and chromosomal DNA to the recipient cells by adhering onto the outer membrane and releasing contents directly into the cytoplasm. A vesicle-associated transformation system has also been reported in several other Gram-negative bacteria, including *E. coli* [24], *H. influenzae* [26], *P. aeruginosa* [27], *N. gonorrhoeae* [28], *Salmonella enterica* [29] and *Ruminococcus* [23].

Transduction, unlike conjugation and transformation, is mediated by bacteriophages (bacteria-infecting viruses), which introduce their own, and sometimes alien, DNA into the host. Transduction can be either 'generalized or common', in which any bacterial gene can be transferred (e.g., as in coliphage P1) or 'specialized or restricted', in which the DNA adjacent to the phage integration is carried as an insertion or substitution in the phage genome (e.g., as in coliphage lambda) and then brought into a new host during infection.

Although bacteriophages require host proteins for replication, the packaging of DNA (whether that of the host or the phage) is solely carried out by phage-encoded proteins. The amount of host DNA that can be packaged within a phage varies greatly and depends on capsid size as well as packaging errors, but it can be up to 100 kilobases [30,31]. The spectrum of infection and organisms that can be transduced by particular bacteriophages depends on the receptor recognition sites present in these viruses. The host range of phages might be a single bacterial species, or a single bacteriophage might be able to infect bacteria from a variety of different phyla. During infection, bacteriophage proteins protect the double-stranded DNA from disintegration by host endonucleases, thereby ensuring safe incorporation into the host chromosome. Bacteriophage can also become dormant (temperate) when integrated into the host chromosome, and many bacterial genomes contain temperate phages or remnants of integrated bacteriophages. Although the infection of bacterial cells is required for bacteriophage replication and propagation, bacteriophage particles survive and are prevalent in the environment [32,33] — in fact, bacteriophage (along with eukaryote-infecting viruses) may constitute the most common source of genetic material on the planet. Therefore, bacteriophage can serve as agents of genetic transfer and introduce new genes into bacterial genomes over broad temporal and spatial scales.

How and Why Sex Evolved in Bacteria

While it is easy to point to the potential benefits of sex and it is known that gene transfer has affected the contents of virtually all bacterial genomes, the origin of sex in asexually reproducing lineages is less clear. It has been argued that each of the mechanisms that currently effect the transfer and uptake of DNA originally served purposes other than sex [34]. It is certainly plausible that transducing bacteriophages might erroneously package and transmit host genes during their normal parasitic life cycle; however, the processes of transformation and conjugation seem to have evolved to transfer and obtain DNA from outside sources as requisite for sex.

Like transduction, conjugation is instigated by a selfish element (a plasmid in this case) and the transfer of host genes might also be ancillary to the overall mission of propagating the plasmid [34]. Also, the development of a competent state, as necessary for transformation, may help to satisfy some nutritional requirements of the cell [35,36]. In addition, it has been proposed that acquired sequences might function in DNA repair [37,38], indicating either that the incoming single-stranded DNA provides erroneous signals of DNA damage or that uptake of DNA itself causes damage. Nevertheless, the uptake of single-stranded DNA might circumvent the activity of host endonucleases, thereby rendering successful recombination. Recently, it has been shown that homologs of the competence genes, which allow the use of extracellular DNA as the sole carbon source, provide an advantage to *E. coli* under conditions of nutrient stress [39], thereby bolstering a nutritional role for competence across a broad range of bacteria. And, after alien DNA has been brought into the cell, it can be recombined into the chromosome using bacterial enzymes that are normally involved in DNA replication and repair.

The Extent and Incidence of Bacterial Sex

The meiotic sex of eukaryotes produces new combinations of genes throughout the entire genome, whereas

Table 1. Genomic fractions of laterally acquired genes (as estimated by compositional characteristics) and levels of recombination (as assayed by MLST) in selected bacterial species.

Species	% Acquired genes[a]	Recombination rate[b]			
		Number of loci tested	Average number of alleles[c]	Polymorphic sites per locus[d]	Recombination level[e]
Bacillus cereus	—	7	32	405	Low
Burkholderia pseudomallei	—	6	19	493	Low
Campylobacter jejuni	3	7	1031	473	Low
Escherichia coli	12–25	12	44	668	Low[f]
Enterococcus faecum	—	7	24	494	Low
Staphylococcus aureus	11–15	7	58	457	Low
Staphylococcus epidermidis	10	7	8	454	Low
Vibrio vulnificus	14	10	34	433	Low
Acinetobacter baumannii	—	7	9	24	Low
Bacillus thuringiensis	—	6	14	475	Low
Haemophilus influenza	4–9	7	34	437	Medium
Streptococcus agalactiae	11–15	7	21	494	Medium
Streptococcus pyogenes	11–18	7	62	448	Medium
Bacillus weihenstephhanensis	—	6	17	475	Medium
Clostridium difficile	—	7	7	377	Medium
Helicobacter pylori	6–9	8	323	481	High
Moraxella catarrhalis	—	8	39	438	High
Neisseria gonorrhoeae	—	15	10	1014	High
Neisseria meningitides	17–22	6	235	466	High
Streptococcus pneumoniae	16–18	7	95	457	High
Pseudomonas aeruginosa	11	7	33	411	High
Oenococcus oeni	—	5	8	456	High
Wolbachia pipiens	—	4	—	—	High

[a] Percentage of laterally acquired genes, obtained from references [52–54,70,71]. Dashes denote unknown values.
[b] MLST data from references [72–78].
[c] Estimated as sum of all alleles/number of loci.
[d] Estimated as sum of all sites/number of loci.
[e] Level of recombination rates based on recombination rate (Low, 0–0.30; Medium, 0.31–0.50; High, 0.51–1.000) or association index (Low, >2.0; Medium, 0.21–2.0, High, <0.2).
[f] See reference [63] for a recent analysis of recombination rates in *E. coli.*

each instance of bacterial sex involves only a small fraction of the genome. Gene transfer can alter bacterial genomes in two ways. Like eukaryotes, there can be an exchange and replacement of homologous regions thereby generating new allelic combinations. (The formation of new genotypes by homologous recombination is what is most commonly referred to as sex in bacteria because its outcome more closely parallels that of higher eukaryotes.) In addition, bacterial sex can also introduce completely novel sequences to the genome (lateral gene transfer) and augment the gene repertoire of the recipient cell. Such acquisition events can result in fundamental changes in the microorganism's lifestyle; for example, the incorporation of pathogenicity islands into benign strains of *E. coli* is sufficient to render them uropathogenic [40], and numerous metabolic pathways and antibiotic resistance genes are now also known to have been acquired by lateral transfer.

Gene transfer resulting in these two types of change to bacterial genomes has been detected by a variety of approaches. Early work examining the gene sequences within a species detected homologous recombination on a very local scale, often acting within genes, within regions on the order of 100 nucleotides in length [41–43]. For the past decade, sequence-based analyses of recombination within species have adopted a multigene approach (multilocus sequence typing, MLST; [44], in which short (400–500 nt) sequences are obtained from a handful of loci distributed around the bacterial chromosome. In this case, the amount of sex (i.e., recombination) among strains is monitored by assessing the assortment of alleles over these loci, with the resulting population structure of species ranging from clonal (no recombination) to panmictic (freely recombining). Initially, MLST studies focused on the genetic diversity harbored by bacterial pathogens of humans [45–48], but this approach has been extended to include bacteria living in diverse environments. Most recently, the availability of complete sequences of several strains within a single bacterial species has led to the analysis of recombination on a genome-wide scale [49–51].

Alternative approaches are employed to identify those segments of a bacterial genome that were acquired by lateral gene transfer [52]. Because such events introduce unique regions into a lineage, newly acquired genes are often restricted to a single genome or have a sporadic distribution among strains. Unfortunately, recognizing the extent of acquired DNA in a genome is not simply a matter of tallying the resident genes that are absent from other genomes, because deletions can yield the same configuration. Phylogenetic methods are sometimes exploited to determine the ancestry of a region (for example, if a gene is present in a closely related outside reference species, then

its distribution is more likely to be attributable to loss in one genome than to acquisition by the other). But one of the most common methods for identifying acquired regions on a genome-wide scale is the examination of the compositional properties of all genes within a genome [53–56]. Because bacterial genomes have characteristic base compositions and codon usage patterns, genes within a genome that were acquired from distant sources have atypical features, and, by this method, it has been estimated that laterally acquired genes constitute up to 30% of some bacterial genomes.

The effective amount of sex — i.e., the amount that is apparent in contemporary gene and genome sequences as opposed to the amount of gene transfer that might actually occur — varies widely across bacterial species. Moreover, there is no tight coupling between the degree to which genes within a species are re-assorted through recombination and the amount of laterally acquired genes within a genome (Table 1). Although the amount of laterally acquired DNA in the *E. coli* and *S. aureus* genomes is fairly high, both species are usually considered to be clonal. In contrast, genome-wide recombination in *H. pylori* is very high despite the relatively low amount of laterally transferred DNA (Table 1).

As additional sequences of complete genomes and more robust phylogenetic and population-based tests of recombination become available, our views about the extent of sex experienced by particular bacterial lineages will become amended. Early protein electrophoretic analyses of *E. coli* reported a relatively high frequency of recombination among strains [57]; however, a refined analysis incorporating several additional loci revealed that the species was essentially clonal, with recombination rates on the order of that of mutation rates [58]. The introduction of nucleotide sequence-based analyses of genetic variation recognized higher levels of recombination both within and across genes, but the population structure of *E. coli* was still viewed as largely clonal because recombination was either limited to particular genes or to particular groups of strains [43,59–62]. In a recent MLST analysis of *E. coli*, almost one-third of the strains were of sufficiently mixed ancestry to prevent their assignment to any of the long-established subgroups within the species [63]. Moreover, this study found a link between sex and virulence, such that pathogenic strains of *E. coli* displayed increased rates of recombination.

Both the presence of a *lac* operon in *E. coli,* but not in its close relative *Salmonella enterica*, and the occurrence of several insertion sequences and prophages suggested early on that several portions of the *E. coli* genome were acquired [64]. When a full genome sequence of *E. coli* became available, base compositional analysis indicated that nearly 18% of its genes arose by lateral transfer [65], an estimate later increased to 25% following consideration of phylogenetic information [66]. Now that genome sequences of several *E. coli* strains have been resolved, the large differences in genome size observed amongst these strains have been shown to be the result of gene acquisition, mostly mediated by bacteriophages [67,68]. In fact, lateral gene transfer has played such a prominent role in the content of bacterial genomes that it appears that vertically transmitted genes (i.e., those genes common to all strains) make up a minor portion of the entire *E. coli* gene pool [68], and that the majority of genes in all bacterial genomes were acquired laterally at some time during the evolutionary history of the lineage [69].

Conclusions

The contents of bacterial genomes are affected by numerous processes, including 'sex', which, as previously mentioned, can be defined in bacteria as the inheritance of DNA from any source aside from their one parent cell. This foreign DNA can be obtained from conspecifics or from distantly related organisms of any domain of life; it can be transferred by cell-to-cell contact, transmitted by any number of infectious agents, or assumed directly from the environment. And, in fact, the sexually acquired DNA need not be integrated or recombined into the bacterial chromosome but can remain as a heritable extrachromosomal element.

Taken together, the features and consequences of sex in bacteria need not overlap with any of those that we normally associate with sex. Unlike the sexual process of higher eukaryotes, bacterial sex does not require the formation or fusion of gametes, cell contact, recombination, or even the creation of genetic variation or new combinations of chromosomal genes, and it is not associated with reproduction. Sex in bacteria is simply the uptake of any genetic material that might eventually be vertically or horizontally transmitted, and now, with the recent availability of complete genome sequences, we have begun to understand its pervasive role in the adaptation and diversification of bacterial lineages.

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