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ScienceDirect
Infection, Genetics and Evolution 8 (2008) 267–285
Infection, Genetics and Evolution
www.elsevier.com/locate/meegid

Adaptive value of sex in microbial pathogens

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Received 18 October 2007; received in revised form 30 December 2007; accepted 2 January 2008
Available online 16 January 2008

Abstract

Explaining the adaptive value of sex is one of the great outstanding problems in biology. The challenge comes from the difficulty in identifying the benefits provided by sex, which must outweigh the substantial costs of sex. Here, we consider the adaptive value of sex in viruses, bacteria and fungi, and particularly the information available on the adaptive role of sex in pathogenic microorganisms. Our general theme is that the varied aspects of sex in pathogens illustrate the varied issues surrounding the evolution of sex generally. These include, the benefits of sex (in the short- and long-term), as well as the costs of sex (both to the host and to the pathogen). For the benefits of sex (that is, its adaptive value), we consider three hypotheses: (i) sex provides for effective and efficient recombinational repair of DNA damages, (ii) sex provides DNA for food, and (iii) sex produces variation and reduces genetic associations among alleles under selection. Although the evolution of sex in microbial pathogens illustrates these general issues, our paper is not a general review of theories for the evolution of sex in all organisms. Rather, we focus on the adaptive value of sex in microbial pathogens and conclude that in terms of short-term benefits, the DNA repair hypothesis has the most support and is the most generally applicable hypothesis in this group. In particular, recombinational repair of DNA damages may substantially benefit pathogens when challenged by the oxidative defenses of the host. However, in the long-term, sex may help get rid of mutations, increase the rate of adaptation of the population, and, in pathogens, may infrequently create new infective strains. An additional general issue about sex illustrated by pathogens is that some of the most interesting consequences of sex are not necessarily the reasons for which sex evolved. For example, antibiotic resistance may be transferred by bacterial sex, but this transfer is probably not the reason sex evolved in bacteria.

Keywords: Recombination; Oxidative stress; Evolution of sex; DNA repair; Microbial pathogens; Bacterial transformation; Multiplicity reactivation

1. Introduction

1.1. Sex is a problem

Sex has two fundamental features: (i) recombination, by which we mean the exchange of genetic information between two homologous chromosomes (usually by physical breakage and exchange), and (ii) outcrossing, by which we mean the chromosomes participating in recombination come from two different individuals. In our usage, ‘‘outcrossing’’ does not necessarily mean ‘‘outbred,’’ outcrossing may be outbred or inbred according to whether the parents are related or not. Recombination is evidently the more basic aspect of sex as indicated by the various reproductive systems that have retained recombination but abandoned outcrossing (e.g., automixis, self-fertilization). On the other hand, there are very few examples of reproductive systems in which outcrossing occurs without recombination. Recombination is fundamental to sex, but it is also a fundamental feature of life, as it occurs in nonsexual stages, such as during mitosis in eukaryotes or after DNA replication before cell division in bacteria. We think that understanding the function of recombination generally will help us understand the function of recombination during sex and so we emphasize in our review those cases for which information on recombination is available.

Although sex is usually thought of as a means of reproduction, this is not always the case. Indeed, in many groups—including viruses, bacteria and most of lower eukaryotes, sex is un-coupled from reproduction; these facultatively sexual species use asexual means for reproduction (e.g., replication, fission, budding, fragmentation) and engage in sex occasionally (in most cases, in response to some form of

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doi:10.1016/j.meegid.2008.01.002

stress). Nevertheless, this type of sex does conform to the two fundamental aspects mentioned above. In bacteria, the process of transformation, involving uptake of homologous DNA from the environment followed by recombination, is a widespread sexual process in nature. Among viruses, recombination between genetically distinct viral genomes during multiple infections of a host cell has been observed for numerous different viruses and is likely widespread in nature. Even in obligately sexual species, a switch to asexual reproduction would be advantageous in terms of strict multiplication, as each individual produces offspring. So, why is sex so widespread?

The principal challenge for any general explanation for the adaptive value of sex is to provide a large enough benefit to balance the large costs of sex. We discuss later the specific costs of sex related to microorganisms, however to motivate the problem of sex we introduce now the major recognized costs of sex. These costs include the cost of males (Maynard Smith, 1978; Williams, 1975), high recombinational load (Shields, 1982) and the costs of mating (Bernstein et al., 1985b). The cost of males applies when males contribute little to the offspring except half the genes (typically in anisogamous species with gametes of different sizes). In these situations, females also contribute half the offspring's genes but they bear the full cost of provisioning the offspring. This yields a 50% cost of sex for females under many conditions. High recombinational load arises from the randomization of genetic information during sex. An organism that has met the test of survival has, by definition, a well-adapted combination of genes. Recombination generates new untested combinations of genes that should, on average, be less successful than the parental combinations. The costs of mating for many organisms are huge, and, according to life-history theory (Stearns, 1992), these investments will detract from other components of fitness.

Another cost associated with sex is the cost for the host of sexually transmitted diseases. In addition to negatively affecting the survival of the host (e.g., HIV, syphilis), pathogens that reside in the host's reproductive system can also directly affect the reproductive potential of the host. Indeed, infection of the male genital tract can cause inflammation (as measured by increased leukocyte concentration in semen) leading to oxidative damage of the DNA within spermatozoa (Aitken and De Iuliis, 2007; Alvarez et al., 2002). This may result in male infertility, poor embryonic development, miscarriage and morbidity of offspring. According to the latest World Health Organization statistics, the estimated number of new cases of syphilis, gonorrhoea, chlamydia and trichomoniasis worldwide for the year 1999 in men and women aged 15–49 years was 340 million new cases (World Health Organization Report, 2001). This amounts to 11% of the world population in this age group. However, this is only a partial picture as there are more than 20 pathogens that are transmissible through sexual intercourse. Sexually transmitted infections (STIs) are thus a major health problem in both industrialized and developing countries. If STIs occurred in a comparable proportion of the population in our primate ancestors or in the ancestors of other sexual species, STIs would have added substantially to the cost of sex in these lineages.

In principle, a switch from obligate or facultative sex to exclusive asexual reproduction would alleviate the above costs. Given the large benefit to be gained from switching, why have so many species retained sex? Presumably, sex provides a sufficiently large benefit to balance these costs. Several classes of theories/hypotheses have been proposed to account for these benefits.

1.2. The variation hypothesis

Evolutionary explanations for sex have usually assumed that the adaptive advantage of sex stems from the genetic variation it produces through crossing over between different loci linked on the same chromosome, through assortment of alleles at different loci on different chromosomes, and through the segregation of alleles at the same locus but on different chromosomes. There are a variety of models and excellent reviews of this active area of research (Barton and Charlesworth, 1998; Otto and Gerstein, 2006; Agrawal, 2006).

A shared feature of all the specific models and of the variation hypothesis generally is that the adaptive value of sex is to be found in its capacity to reduce the statistical genetic associations between alleles when these associations are interfering with selection. Genetic associations may arise by chance such as in finite populations (Hill and Robertson, 1966; Otto and Barton, 2001) or by epistatic interactions of the effects of alleles on fitness (Otto and Feldman, 1997). Epistasis means that the fitnesses of the extreme genotypes are different from what is predicted from the intermediate genotypes. Positive epistasis selects for reduced recombination while negative epistasis may select for increased recombination, but only if the negative epistasis is weak and not too variable across pairs of loci (Barton, 1995; Otto and Feldman, 1997). When epistasis is too negative, the evolution of a modifier of recombination is dominated by its direct fitness cost of breaking apart the genetic associations that have been built by epistatic selection (recombinational load).

A particular form of negative epistasis occurs in the mutational deterministic model which concerns the capacity of sex to get rid of deleterious mutations in large populations (Kondrashov, 1988). Negative epistasis of mutations means that the fitness of an individual bearing two or more deleterious mutations is less than the product of the fitnesses of individuals bearing the corresponding single deleterious mutations. Sex may be advantageous by bringing deleterious mutations together in individual organisms that are then more effectively removed by natural selection. Data from viruses discussed below cast doubt on the generality of negative epistasis of mutations.

Another form of epistasis is based on Red Queen coevolution (Bell, 1982) and one particular form of the Red Queen hypothesis postulates that the main selective force maintaining sex is protection against parasites and pathogens (Hamilton, 1980; Seger and Hamilton, 1988). Population genetic analysis of this model has shown that the epistasis generated by host–parasite interactions is generally too strong and selects against sex (Otto and Nuismer, 2004). Host–parasite

interactions may also generate fluctuating epistasis which selects for sex, but only if the fluctuations are rapid (Barton, 1995; Gandon and Otto, 2007). Data from pathogens bearing on the host–parasite model will be discussed below.

Because of these and other problems with the negative epistasis model, some workers in this area favor selection and chance effects in finite populations as the main sources of genetic associations that may provide an advantage for sex (Otto and Gerstein, 2006; Peters and Otto, 2003). According to this view, advantageous mutations occurring on advantageous backgrounds will sweep through the population. Disadvantageous mutations on disadvantageous backgrounds will be quickly eliminated. However, when advantageous mutations occur on chromosomes with disadvantageous alleles (or vise a versa), selection is stalled. Such mixed chromosomes with both advantageous and disadvantageous alleles tend to accumulate in the population and produce negative linkage disequilibrium which may select for increased recombination under certain conditions. The increased response during artificial selection of sexual populations as compared to asexual populations has been viewed as support of this model (Peters and Otto, 2003).

1.3. The DNA repair hypothesis

We have proposed that the evolution of sex is a consequence of coping with the two main sources of error in the transmission of genetic information: genetic damage (i.e., DNA or RNA damage depending on the nature of the genome) and mutation (Bernstein et al., 1985a). Damages and mutations are both genetic errors but they differ in fundamental ways. Damages are physical irregularities in the genetic material, usually DNA, and may be recognizable at the structural level. Because they can be recognized directly in the DNA they may be correctly repaired if there is redundant information available. Mutations are changes in the base sequence of the genetic material and are not recognizable at the DNA level, once the base changes are present in both strands. Mutated DNA is still a regular DNA double helix. Mutations may be replicated and passed on to offspring and will increase or decrease in frequency in the population according to chance events and their effects on fitness. The vast majority of non-neutral mutations are deleterious, although advantageous mutations occur. Advantageous mutations are often observed in viruses and bacteria that characteristically exist in large populations. In contrast to the heritability of mutations, damages usually interfere with replication and are not passed on to offspring cells or to progeny. In short, damages may be recognized and repaired, but not replicated and inherited, while mutations are not recognized and repaired, but are replicated and inherited.

Damages and mutations are related because damages can cause errors of DNA synthesis during replication or repair and these errors are the predominant source of mutations. There is increasing evidence that most spontaneously occurring mutations are a result of previously existing damages in the DNA. Unrepaired damages are converted to mutations by error-prone bypass of the damage by the polymerase, sometimes referred to in the literature as translesion synthesis (Kunz et al., 1998). In cases where it was possible to determine the cause of mutations, lesion bypass was the most frequent category. For example, in yeast at least 60% of spontaneous single base pair substitution and deletion mutations are caused by synthesis past DNA damages in the template strand (Stuart et al., 2000; Stuart and Glickman, 2000). In studies of lacI spontaneous mutations in different organs in transgenic mice of different ages, most mutations are caused by the translesion synthesis of premutagenic lesions (Stuart and Glickman, 2000).

We have argued that the primary adaptive value of recombination is the accurate and efficient repair of genetic damage so that the genome(s) transmitted to progeny are free of damage and mutations. By ‘‘primary adaptive value’’ we mean to indicate the dominant effect on fitness in the short-term. There may be other effects, indeed, with a trait like sex which is so entrenched in the biology of life we expect there to be a variety of other significant effects. The reduction of genetical associations between alleles that can result from recombination is certainly among these significant effects. However, we note that often sex does not have this effect, because the chromosomes in the mates can be identical and the recombination cryptic from the point of view of affecting statistical associations of alleles. Indeed, we will argue that this is often the case in the life-cycle of many pathogens. We feel that not explaining the ubiquity of cryptic recombination is a limitation of the variation hypothesis.

The evidence that recombination evolved as an adaptation for DNA repair is substantial in a variety of organisms of differing levels of complexity in the hierarchy of life (Bernstein et al., 1987; Bernstein and Bernstein, 1991; Birdsell and Wills, 2003; Michod and Gayley, 1994; Cox, 2001; Michod, 1995). There are five kinds of evidence. The first three kinds of evidence are reviewed in Bernstein and Bernstein (1991). (i) Mutations in recombination genes directly and immediately lower fitness by making cells sensitive to DNA damage. (ii) Increased levels of a DNA damaging agent increase the rate of recombination. (iii) Mutations in other DNA repair genes further increase the rates of damage-induced recombination. (iv) Diploid cells are more resistant to DNA damage than haploid cells (Herskowitz, 1988; Zirkle and Tobias, 1953; Game, 1983). (v) Finally, the recombination systems in eukaryotes and prokaryotes are evolutionarily conserved in that there is a continuous evolution of the recombination system as a DNA repair system (Cromie et al., 2001; Lin et al., 2006; Ramesh et al., 2005).

Giardia intestinalis, an enteric protozoan parasite was regarded as a primarily asexual eukaryote. Nevertheless, the *G. intestinalis* genome has several core meiotic genes that are also widely present among sexual eukaryotes (Ramesh et al., 2005). Some of these genes are also homologous to bacterial genes necessary for recombinational repair (e.g., *dmc1* of *G. intestinalis* is a homolog of *Escherichia coli recA*, discussed below). These findings indicate that meiosis was probably present early in eukaryote evolution, and evolved from recombinational repair-related processes in bacteria. *G. intestinalis* was also recently found to undergo recombination and thus presumably sexual reproduction, which argues for the

continuity of sex and recombinational repair across the prokaryotic–eukaryotic border (Cooper et al., 2007).

Several types of DNA damages have been described (reviewed in Slupphaug et al., 2003). Single-strand damage may be repaired using information from the complementary strand in a single DNA double helix. Double-strand damages are more difficult to repair as they involve loss of information in both strands and require recombination involving a homologous chromosome. A common type of double-strand damage is the double-strand break (DSB). For humans, the spontaneous rate of endogenous DSBs is about 50 per cell per cell cycle (Vilenchik and Knudson, 2003). Since a single unrepaired DSB can block replication and be lethal to a cell, the importance of recombinational repair, the only repair process that can accurately repair DSBs, is apparent.

Life depends on a delicate balance between reduction and oxidation reactions. Various metabolic or environmental factors (i.e., stress factors) can upset this balance, leading to an increase in the cellular levels of reduced and highly reactive molecules, including a series of oxygen-containing compounds collectively termed reactive oxygen species (ROS). ROS include the superoxide anion, O_2^-; the hydroxyl radical, OH; and hydrogen peroxide, H_2O_2. At high concentrations, ROS can be damaging to biological systems (i.e., oxidative stress), and can lead to DNA damage, including double-strand damage. Thus, under oxidative stress, the number of DSBs, and the need for their efficient repair, would increase substantially. As most types of stress result in an increase in the cellular levels of ROS and as sex in facultatively sexual lineages is induced by stress (e.g., nitrogen deprivation, heat-stress), we hypothesized that sex evolved as a response to oxidative stress and its DNA damaging effects (Nedelcu and Michod, 2003; Nedelcu et al., 2004). Consistent with this hypothesis, in two facultatively sexual microorganisms, the yeast *Schizosaccharomyces pombe* and the green alga, *Volvox carteri*, sex is induced by oxidative stress (Bernstein and Johns, 1989; Nedelcu and Michod, 2003; Nedelcu et al., 2004).

While it is clear that DNA damage is frequent and that recombinational repair is important for dealing with double-strand damage, it is not clear why recombination is often, although by no means always, associated with outcrossing. It is the organization of recombination in the life-cycle of organisms that is difficult to understand solely on the basis of the need for DNA repair. This is especially true in predominantly diploid organisms, since in principle they have the genetic redundancy needed for repair available in most cells most of the time. However, the redundancy provided by diploidy is likely more effective for repair during meiosis when the rate of recombination is several orders of magnitude higher than during mitosis. Indeed, meiosis seems designed not only for effective recombination but for effective recombinational repair (Bernstein et al., 1988).

In predominantly haploid organisms, the subjects of this review, the question of the advantage of outcrossing from the point of view of genetic repair is not so complicated, as outcrossing serves to create the diploid state necessary for recombinational repair. Of course, diploidy need not only be created by fusion, it may also be created within the cell by DNA replication without cell division. For example, recombinational repair may occur between sister chromatids as in the repair of stalled replication forks (Cox et al., 2000). Nevertheless, while useful for repairing certain kinds of damages, such a within cell strategy of creating diploidy cannot cope with double-strand damages present before the replication of the chromosome. If diploidy is important for the recombinational repair of double-strand DNA damages, then there is the question of why not just stay diploid, why return to the haploid state. The answer appears to be that effective DNA repair is not the only component of fitness and, in certain environments, haploids have advantages over diploids in these other components (Cavalier-Smith, 1978; Mable and Otto, 1998; Otto and Marks, 1996; Long and Michod, 1995; Destombe et al., 1993; Perrot et al., 1991; Valero et al., 1993; Adams and Hansche, 1974). For instance, while diploid cells are better able to survive DNA damaging environments, haploid cells are more efficient replicators and so may be favored in relatively benign environments (Long and Michod, 1995).

1.4. Theme and approach of review

We consider the adaptive value of sex in viruses, bacteria and fungi, and particularly the information available on the adaptive role of sex in pathogenic organisms. Our general theme is that the varied aspects of sex in pathogens illustrate the varied issues surrounding the evolution of sex generally. However, this is not a general review of the evolution of sex in all organisms. Rather, we specifically consider the adaptive value of sex in microbial pathogens. There are costs of sex, both to the host and to the pathogen. There are short-term and longer term consequences, and possible benefits, of sex. We consider three general hypotheses for the adaptive value of sex. These hypotheses are (i) sex provides for effective and efficient recombinational repair of DNA damages, (ii) sex provides DNA for food, and (iii) sex produces variation and reduces genetic associations among alleles under selection. We conclude that in the short-term the DNA repair hypothesis has the most support and is the most generally applicable hypothesis in pathogens. However, in the long-term, sex may help get rid of mutations, increase the rate of adaptation of the population, and in pathogens may infrequently create new infective strains. A final general issue about sex illustrated by pathogens is that some of the most interesting consequences of sex are not necessarily the reasons sex evolved. For example, antibiotic resistance may be transferred by bacterial sex, but this transfer is probably not the reason sex evolved in bacteria.

In almost every discussion of the problem of the evolution of sex, it is noted that there are too many theories and few experimental and empirical systems in which these theories can be tested (e.g., Barton and Charlesworth, 1998; Agrawal, 2006). Consequently, we have adopted the strategy in our review of focusing on the experimental data available in pathogenic viruses, bacteria and fungi. First, we consider sex in bacteriophage T4 and several human pathogenic viruses. Then we review evidence on bacterial pathogens and fungal

pathogens. We then discuss the overall balance of benefits and costs of sex in microbial pathogens. Finally, we evaluate what we have learned about the evolution of sex more generally as a result of considering sex in infectious pathogens.

2. Viral pathogens

2.1. Overview

In this section, we discuss pathogenic viruses in which sex, in the form of recombination between co-infecting viruses, has been studied. These viruses are (i) the model system bacteriophage (phage) T4 which infects *E. coli*, and (ii) the human pathogens, influenza virus, human immunodeficiency virus (responsible for AIDS), and Herpes simplex virus. In each case, we review evidence concerning the adaptive function of sexual recombination. In viruses, recombinational repair is most often studied as it is manifested in the phenomenon of multiplicity reactivation (MR). MR is the process by which viral genomes containing inactivating genomic damage interact within the infected cell to form a viable genome.

Ordinarily, MR is determined by measuring survival of viral infectivity using a plaque-forming assay after treatment of a virus suspension with increasing doses of an agent that damages the DNA or RNA genome. Both multiply infected cells and singly infected cells are scored for plaque-forming ability. The results shown in Fig. 1 are redrawn from Chen and Bernstein (1987) where the DNA damaging agent used was H_2O_2. A noteworthy feature of this type of result is that at doses of agent which reduce survival of the singly infected cells by more than an order of magnitude, the survival of the multiply infected cells is reduced by only a small amount. Multiple infections

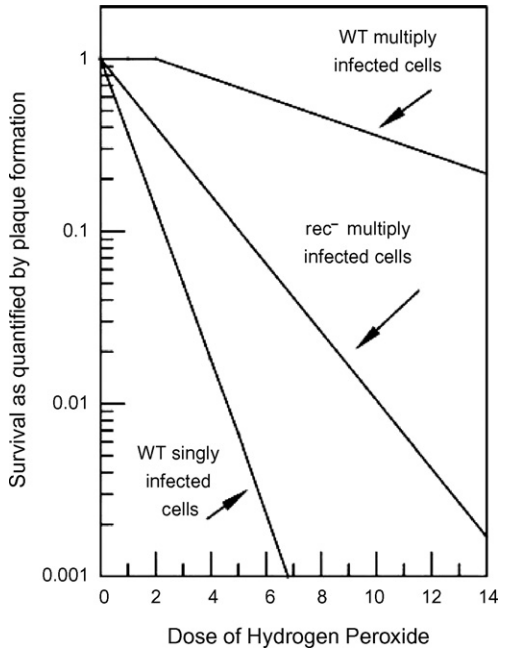

Fig. 1. Inactivation curves of plaque forming ability of cells singly infected and multiply infected by phage T4 versus dose of H_2O_2. Results are shown for multiple infection by wild-type (WT) phage and by tsL67 mutant phage defective in recombination (rec$^-$).

carried out with a temperature-sensitive mutant (tsL67), indicated as rec$^-$ in the figure, at a semi-permissive temperature showed substantially less MR than wild-type multiple infections. This mutant is defective in a DNA single-stranded binding protein that is necessary for recombination. Temperature-sensitive mutants defective in other proteins necessary for recombination showed similar reductions in MR. These proteins included an exonuclease and the product of gene *uvsX* (a homolog of *E. coli recA*). Among viruses that use MR, multiple infection may provide a substantial selective benefit. Below, we argue, based on experimental data as well as specific aspects of infection in several pathogenic viruses, that the short-term benefit of MR is recombinational repair. In the last section, we present other proposed benefits of MR and sex in viruses.

2.2. Phage T4

Phage T4, a DNA virus that infects *E. coli*, serves as a model for understanding MR (Bernstein and Bernstein, 2001; Bernstein, 1981, 1987; Bernstein and Wallace, 1983). MR is considered to be a recombinational repair process on the basis of the following: (1) MR requires at least two chromosomes. (2) For phage T4 damaged by UV, nitrous acid, mitomycin C or H_2O_2, MR depends on several gene functions required for normal levels of spontaneous recombination (e.g., Gp32, Gp46, Gp47 and UvsX) (Bernstein and Wallace, 1983; Chen and Bernstein, 1987). (3) Under conditions where MR occurs, the frequency of genetic recombination increases. H_2O_2 and its free radical product, the hydroxyl radical ($OH^\bullet$) are ubiquitous causes of DNA damage in living organisms. The lethal damages introduced by H_2O_2 in phage T4 were repaired by MR more efficiently than most other types of damages studied (Chen and Bernstein, 1987), suggesting that recombinational repair efficiently removes oxidative damages.

The enzymes employed in recombinational repair in phage T4 are functionally homologous to enzymes employed in bacterial and eukaryotic recombinational repair (including human meiotic recombinational repair) (Bernstein and Bernstein, 2001; Cromie et al., 2001; Li et al., 2007). In particular, there is functional homology from viruses to humans in a gene required for two key steps of recombinational repair. These steps are homologous DNA pairing and the strand exchange reaction between homologs. The gene is known as *uvsX* in phageT4, as *recA* in *E. coli* and other eubacteria, and as *rad51* and *dmc1* in yeast and other eukaryotes including humans (Cromie et al., 2001; Lin et al., 2006). The gene *dmc1* is specifically required for meiotic recombination in yeast, plants and animals (Lin et al., 2006). The existence of this and other homologies in the proteins of the DNA recombinational repair machinery imply continuity in the evolution of sexual processes from microorganisms to humans.

2.3. Influenza virus

The influenza viral genome is composed of eight physically separate single-strand RNA segments. Influenza virus has been shown to undergo MR after inactivation by UV irradiation

(Barry, 1961; Henle and Liu, 1951), and by ionizing radiation (Gilker et al., 1967). If any of the segments is damaged in such a way as to prevent replication or expression of an essential gene, the phage is inviable when it, alone, infects a cell (a single infection). However, when two or more damaged viruses infect the same cell (multiple infection), the infection can succeed provided each genomic segment is present in at least one undamaged copy, i.e., MR occurs.

Upon infection, influenza virus induces oxidative stress and increased ROS production as a host response (Peterhans, 1997). If oxidative damage is a consistent problem for virus survival under natural conditions, then MR will be selectively advantageous and this simple form of sex will be maintained. We have suggested that MR involving segmented RNA genomes may be similar to the earliest evolved sexual processes when organisms with RNA genomes predominated and the source of DNA damage was UV stress (Bernstein et al., 1984).

2.4. Human immunodeficiency virus type 1 (HIV-1)

HIV-1 encapsidates two RNA genomes in each viral particle and during the next replicative cycle, catalyzed by reverse transcriptase, recombination between the two genomes can occur (e.g., Charpentier et al., 2006; Hu and Temin, 1990). Recombination occurs during the minus-sense DNA synthesis step of reverse transcription. The nascent DNA can transfer multiple times between the two copies of the viral genomic RNA (Lanciault and Champoux, 2006). This type of recombination is referred to as copy-choice. Copy-choice events occur throughout the genome at a frequency of 2–20 events/genome/replication cycle, and these have the ability to rapidly shuffle genetic information from parental viruses in transmission to progeny genomes (Charpentier et al., 2006). Switching of the reverse transcriptase tends to occur at pause sites (inverted repeat sequences that form stem-loop structures; Lanciault and Champoux, 2006) and at sites of nucleotide misincorporation (Diaz and DeStefano, 1996; Palaniappan et al., 1996).

What is the adaptive advantage of this type of recombination? Viral recombination appears to contribute to the evolution of resistance to anti-retroviral therapy (Nora et al., 2007). Viral recombination, in principle, may also contribute to overcoming the immune defenses of the host. Nevertheless, for the advantages of allelic variation to be realized, the two copies of the viral genome packaged in the HIV particle need to be from separate parental viruses of differing genetic constitution. However, it is currently unknown how frequently such mixed packaging occurs under natural conditions (Chen et al., 2006). On the other hand, the advantage of recombinational repair applies whether or not the genomes have allelic differences.

It is interesting that infection by HIV-1 causes chronic ongoing inflammation and production of ROS (Israel and Gougerot-Pocidalo, 1997). Thus, the virus may be subject to oxidative stress leading to damages, including breaks in the ssRNA genome. Bonhoeffer et al. (2004) suggested, as an explanation for the evolution of recombination in HIV, that template switching by the reverse transcriptase could act as a repair process to deal with breaks in the ssRNA genomes. Hu and Temin (1990) also suggested that recombination in HIV may be an adaption for repair of damage to the RNA genomes. During reverse transcription, strand switching (copy-choice recombination) could generate an undamaged copy of genomic DNA from two damaged ssRNA copies. This could explain why retroviruses carry two complete genomes. According to this explanation, allelic recombination in retroviruses would be a consequence, but not the cause of, the evolution of template switching (Bonhoeffer et al., 2004). The recombination could be a mechanism for dealing with the host induced oxidative damage.

2.5. Herpes simplex virus (HSV)

HSV is a double-stranded DNA virus that can establish a long-term latent or persistent infection. When HSV particles that have received DNA damage are allowed to undergo multiple infection of human cells, MR is observed. For instance, HSV undergoes MR in response to lethal DNA damages caused by methyl methanesulfonate, MNNG (Das, 1982), trimethylpsoralen (which causes inter-strand cross-links) (Hall and Scherer, 1981; Coppey et al., 1989), and UV light (Selsky et al., 1979). Recombination between genetically marked pairs of herpes viruses was increased after treatment of the parental viruses with trimethylpsoralen, suggesting that psoralen damage stimulates genetic recombination (Hall and Scherer, 1981). MR of HSV depends, at least in part, on the host cell recombinational repair machinery since skin fibroblast cells defective in the Bloom's syndrome gene, a component of this machinery, are defective in MR (Selsky et al., 1979). These findings indicate that MR in this virus involves genetic recombination between damaged viral genomes leading to production of viable progeny virus particles.

Interestingly, HSV-1 infection is also associated with oxidative stress in the host cells (Valyi-Nagy et al., 2000). Thus, double-strand DNA breaks may arise as a consequence of replication fork collapse at sites of oxidative damage, and recombination may be involved in repairing these double-strand breaks (Nimonkar and Boehmer, 2003).

2.6. Other studies of sex in viruses

In addition to the studies of MR in pathogenic viruses described above for influenza virus, HIV, and HSV, MR has also been reported for adenovirus (Yamamoto and Shimojo, 1971; Day et al., 1975), simian virus 40 (Yamamoto and Shimojo, 1971; Hall, 1982), vaccinia virus (Abel, 1962), reovirus (McClain and Spendlove, 1966) and poliovirus (Drake, 1958). Similarly, in addition to the studies of MR described above for phage T4, MR has also been studied in other phages. Phage lambda, which infects *E. coli* undergoes MR after UV treatment (Huskey, 1969). In this case, MR depends either on the host RecA protein, or on a phage recombination protein, Red. The absence of both gene products results in loss of MR (Huskey, 1969), indicating, again, that MR is a recombinational repair

process. MR has also been reported in other phages that infect *E. coli* (T1, T2, T5, T6, and phiX174) and *Salmonella typhi* (Vi-phage) (see Bernstein, 1981 for references). Lastly, studies of cauliflower mosaic virus as a model have shown that recombination resulting from co-infection is very frequent in the everyday life of the virus (Froissart et al., 2005). Altogether, these findings indicate that MR is a widespread feature of sex in viral pathogens, and that, in the short-term, selection for sex in these viruses is likely based on the benefits of recombinational repair upon multiple infection.

2.7. Other proposed benefits of MR and sex in viruses

In addition to its immediate, short-term benefit of promoting virus survival and reproduction, MR can also influence viral evolution by generating viral allelic variation. Generally, the optimal epistatic interactions among genes are likely to be broken by recombination, and most non-neutral recombinants, like most non-neutral point mutations, are likely to be deleterious. However, recombination allows some viruses to acquire key adaptive mutations in a single step and hence to make a major jump in fitness space. This may allow infectious sweeps through the human population (Scholtissek, 1995). Nevertheless, since this beneficial effect of recombination is a rare occurrence in the overall virus population, it is likely that the immediate selective benefit that maintains sex in the viral generations between major jumps in fitness is the ability to overcome genomic damage, allowing the virus to survive and reproduce.

Another possible advantage of sex, in the long-term, was reported for phage $\phi 6$ that has a genome composed of three RNA segments (Poon and Chao, 2004). In response to selection for growth at high temperature on its host bacterium *Pseudomonas phaseolicola*, sexual lines (multiple infections) on average outperformed asexual lines (single infection). However, the advantage of sex was attenuated with increasing population size. This indicated to the authors that the rate of adaptation in the asexual lines was limited in smaller populations by interference among the genomic segments whose associations were determined by genetic drift. Sexual recombination, by generating the fittest genotypes, increases genetic variation in fitness and hence the rate of response to selection. Noteworthy, in the analysis of these results, a possible effect of MR was not considered.

Recently, HIV-1 was used as a model to investigate the mutational deterministic model of sex discussed in the introduction. Under this model, for sex to be maintained, deleterious mutations need to interact with negative epistasis, so that the fitness of an individual bearing two or more deleterious mutations is less than the product of the fitnesses of individuals bearing the corresponding single deleterious mutations. However, epistatic interactions in HIV-1 proved to be positive, rather than negative (Bonhoeffer et al., 2004; Michalakis and Roze, 2004). In studies of epistatic interactions in several additional organisms, epistatic interactions also proved to be positive (or absent) (Kouyos et al., 2007). These findings, not only failed to support the mutational deterministic model, but unexpectedly indicated that sex should be selected against if epistatic interaction between deleterious mutations is a predominant selective force.

3. Bacterial pathogens

3.1. Overview

Three processes involving homologous gene transfer are recognized in bacteria. These are plasmid-mediated conjugation, phage-mediated transduction, and natural bacterial transformation. Plasmid-mediated conjugation is controlled by plasmid genes, and is an adaptation for spreading copies of the plasmid between bacteria. The infrequent integration of a plasmid into the host bacterial chromosome, and the subsequent transfer of part of the host chromosome to another cell, does not appear to be a bacterial adaptation. Likewise phage-mediated transduction of bacterial genes likely reflects an infrequent mistake in the assembly of the phage particle, rather than a bacterial adaptation.

In contrast, bacterial transformation is a complex process encoded by numerous bacterial genes and is clearly a bacterial adaptation for DNA transfer. Transformation involves the transfer of naked DNA from one bacterium to another through the surrounding medium. Transformation occurs naturally in a wide range of at least 40 different bacterial species (Hudson and Michod, 1992; Lorenz and Wackernagel, 1994). In some species, like *Bacillus subtilis*, non-homologous DNA may be brought in, while in other species, like *Hemophilus influenza*, only homologous DNA is taken-up. In both cases, however, only homologous transforming DNA is ordinarily incorporated into the bacterial chromosome by recombination. However, with low frequency, homologous recombination can effect transfer of heterologous genes into a recipient by homology-facilitated illegitimate recombination (Majewski and Cohan, 1999). Transformation is an adaptation, rather than an incidental trait, since it results from a complex, energy-requiring developmental process. For a bacterium to bind, take up, and recombine exogenous DNA into its chromosome, it must enter a special physiological state referred to as competence. Transformation involves expression of numerous genes necessary for competence and recombination of DNA, and appears to be the primary form of adaptive gene transfer in bacteria.

Transformation has been studied in the human pathogenic bacteria *Neisseria gonorrhoeae*, *Hemophilus influenzae*, *Streptococcus pneumoniae*, *Streptococcus mutans* and *Helicobacter pylori*, but the significance of transformation for bacterial pathogenicity is not well understood. Here we will focus on the adaptive function of transformation, emphasizing evidence from the model organism *B. subtilis*, and from the pathogens mentioned above. In addition to the pathogenic bacteria described below, several additional bacterial pathogens are capable of transformation. These include *Campylobacter jejuni*, *Campylobacter coli*, *Hemophilus parainfluenzae*, *Neisseria meningitides*, *Staphylococcus aureus* and *Streptococcus sanguis* (see Lorenz and Wackernagel, 1994 for references).

Below, we present evidence to support the short-term benefit of bacterial transformation as a DNA repair mechanism. In the last section, we discuss other proposed hypotheses for the adaptive role of sex in bacteria.

3.2. *B. subtilis*

Transformation and recombination in *B. subtilis* depend on the *recA* gene which is a homolog of the well-studied *recA* gene of *E. coli* (Alonso et al., 1991). Expression of the *B. subtilis recA* gene is induced following DNA damage and during the development of the competent state (Cheo et al., 1992). RecA protein is an integral part of a multiprotein assembly along with several competence proteins. This assembly accepts incoming ssDNA at a cell pole, where it is prepared for recombination with the chromosome (Kidane and Graumann, 2005). The incoming DNA and RecA protein form striking filamentous structures, which emanate from the pole containing the competence machinery and extend into the cytosol. These RecA threads are thought to represent dynamic RecA/ssDNA nucleofilaments that scan the chromosome for regions of homology, and thus bring incoming DNA to the corresponding site in the nucleoid where strand exchange occurs (Kidane and Graumann, 2005). Cox (1991, 1993, 2001) has reviewed evidence that RecA protein is adapted for recombinational repair rather than the generation of allelic variation. For instance, Cox (1993) noted evidence that RecA protein binding is largely limited to regions in duplex DNA containing suitable nucleation sites, and that a variety of DNA damages cause structural perturbations that provide favorable nucleation sites.

At least 40 genes are required for competence in *B. subtilis* (Solomon and Grossman, 1996). In culture, cells typically become competent at transformation during the late-log phase of culture growth in which most cells are haploid. The association of competence with haploidy is adaptive in terms of the need for genetic template for use in recombinational repair. During the log phase of growth many cells have two or more copies of the chromosome and post-replication recombinational repair is a viable strategy for many damages.

According to the repair hypothesis, it is expected that transformation adaptively responds to levels of DNA damage and that the exogenous DNA taken-up by cells is used as template for recombinational repair of damages in the recipient cell's genome. When derived from protoplast lysates, the length of continuous DNA incorporated into *B. subtilis* can be greater than 1271 kbases, which corresponds to about one-third of the whole-genome length (Saito et al., 2006). Evidence has also been presented that the whole chromosome from protoplast lysates is incorporated into competent cells (Akamatsu and Taguchi, 2001). The finding that DNA as large as 1/3 to all of the genome can be transferred, indicates that there is a good chance that homologous template will be available to allow recombinational repair of any randomly situated damage in the recipient DNA.

The adaptive function of bacterial transformation has been studied in *B. subtilis* as a model system for understanding the function of transformation and, even more generally, the evolutionary function of sexual recombination (Hoelzer and Michod, 1991; Michod et al., 1988; Michod and Wojciechowski, 1994; Wojciechowski et al., 1989). Transforming DNA was administered to cultures grown to maximal competence either before (''DNA-UV'') or after (''UV-DNA'') exposing the cells to UV radiation, a known DNA damaging agent. Oxidative stress was also used as a damaging agent in the form of H_2O_2 and results similar to those described below with UV were observed (Michod and Byerly, unpublished data). Three kinds of donor DNA were utilized, homologous chromosomal DNA containing an auxotrophic marker, non-homologous plasmid DNA with a non-homologous marker, and plasmid DNA containing a short fragment of homology at an auxotrophic marker locus (usually the trp^+ locus). Transformants at the marker locus must be sexual cells, while the total population of cells, even in a culture grown to maximal competence, is composed primarily of non-transformed asexual cells (as under the best of conditions approximately 10% of the cells in the culture become competent; Dubnau, 1982). So we regarded the comparison of transformed cells versus total cells as a comparison of sexual versus asexual cells, respectively.

''Survival'' of transformed (sexual) cells and total (asexual) cells in a UV-damaging environment was measured by the density of the two kinds of cells at a specific UV dosage divided by the density of the same kind of cell not exposed to UV. It was found that transformed (sexual) cells survived UV better than total (asexual) cells, but only in UV-DNA experiments in which the donor DNA was administered after the UV treatment (Michod et al., 1988). The increase in survival was observed even if the donor DNA was irradiated with UV at levels comparable to that given to the recipient cells (Hoelzer and Michod, 1991). This is the expected result if DNA damages are induced randomly, and donor DNA preferentially participates in recombinational repair at sites of DNA damage in the recipient chromosome. These results support a prediction of the DNA repair hypothesis that transforming DNA confers a repair benefit on damaged cells.

However, the apparent enhanced survival could also be explained by another prediction of the repair hypothesis that the transformation (sex) should be increased in DNA damaging environments. If this were the case, the transformation rate might be higher at higher levels of DNA damage, resulting in a higher measured survival of transformed cells. Of course, both processes could be occurring simultaneously and likely are. Probably there is already exogenous DNA present in a competent culture, even when no external transforming DNA is added, as competent cells actively export DNA (Streips and Young, 1974; Crabb et al., 1977; Sinha and Iyer, 1971; Lorenz and Wackernagel, 1994). Noteworthy, in some bacterial species transformation is coupled with autolysis, which releases into the environment DNA that is picked up by the surviving cells (see Section 3.5).

In *B. subtilis*, experiments were also conducted using, as transforming DNA, non-homologous plasmid DNA and plasmid DNA carrying a short gene-sized sequence of marked chromosomal DNA (Michod and Wojciechowski, 1994;

Michod et al., 1988). Both homologous DNA and non-homologous plasmid DNA is taken-up by competent cells. We observed no increase in transformation with UV dosage when using non-homologous donor plasmid DNA but a significant increase when using plasmid donor DNA containing a short cloned sequence of marked chromosomal DNA. These results were interpreted to mean that the increase in survival of transformed cells with UV dosage in our experiments was primarily due to homologous transformation being inducible by DNA damage (Michod and Wojciechowski, 1994; Michod et al., 1988).

What might be the mechanism for the DNA damage inducibility of transformation? As the cultures were grown to maximal competence, the recombination system is maximally induced in competent cells (Lovett et al., 1989). Michod and Wojciechowski (1994) hypothesized that this transformation induction did not involve the induction of competence nor the induction of the RecA protein, but rather involved activation of the RecA protein leading to the increased binding of RecA to incoming single-stranded DNA and the promotion of the RecA-mediated strand exchange between these incoming molecules and regions of gapped DNA in the recipient chromosome (Roca and Cox, 1990). There are, of course, other proteins involved in homologous recombinational repair in addition to RecA, such as helicases, resolvases, etc., and these other proteins may also be activated by UV irradiation.

In summary, we think that the observed increase in transformation of marked donor DNA after UV irradiation reflects: (1) activation of the RecA protein, and/or other proteins involved in recombination, by the introduced DNA damages in the recipient DNA, and (2) homologous recombinational repair of these damages utilizing a mixture of the added marker DNA and the DNA naturally present because it is excreted by surrounding competent cells.

3.3. N. gonorrhoeae

Infection by *N. gonorrhoeae* (gonococci), a strictly human pathogen, causes severe exudative urethritis. The exudates from infected persons contain large numbers of polymorphonuclear leukocytes (PMN) with ingested gonococci. *N. gonorrhoeae* stimulates PMN to produce an oxidative burst involving ROS (Simons et al., 2005). These ROS are produced predominantly inside the PMN in response to the gonococci. A significant portion of the gonococci is able to resist killing and are able to replicate within PMN phagosomes in spite of the respiratory burst.

Stohl and Seifert (2006) presented evidence that recombinational DNA repair mediated by RecA protein plays an important role in *N. gonorrhoeae* in protecting against oxidative DNA damage, although they did not focus specifically on recombinational repair during transformation. However, this finding together with the evidence, reviewed above in *B. subtilis*, that transformation promotes recombinational repair mediated by a RecA protein homologue, suggests that in *N. gonorrhoeae*, transformation also has a recombinational repair function. This inference is supported by recent evidence on the specificity of DNA uptake during transformation, discussed below.

DNA uptake during transformation in *N. gonorrhoeae* requires short DNA sequences (9–10mers residing in coding regions) in the donor DNA. These sequences are referred to as DNA uptake sequences (DUSs), and they appear to be conserved among phylogenetically divergent species. DUS occur with a significantly higher density within genes involved in DNA repair and recombination (as well as in restriction-modification and replication) than in any other annotated gene group in these organisms (Davidsen et al., 2004). Davidsen et al. (2004) suggested that the overrepresentation of DUS in DNA repair genes may reveal the benefits of maintaining or restoring the integrity of the repair machinery through preferential uptake of genome maintenance genes that are particularly important and must be replaced by new copies if irreparably damaged or lost. Taken as a whole, the evidence suggests that an important benefit of transformation in *N. gonorrhoeae* is recombinational repair of oxidative DNA damages caused by oxidative attack by the host's phagocytic cells.

3.4. H. influenzae

In the completely sequenced genome of *H. influenzae*, at least 15 genes are involved in transformation (Fleischmann et al., 1995). As in *N. gonorrhoeae*, discussed above, DNA uptake during transformation in *H. influenzae* requires DUS which occur with a significantly higher density within genes involved in DNA repair, recombination, restriction-modification and replication (Davidsen et al., 2004).

Transformation in *H. influenzae* depends on the *rec1* gene that is a homolog of the *E. coli recA* gene. *rec1* mutants are defective in recombination and DNA repair (Setlow et al., 1988). Another *H. influenzae* gene, *rec2*, has roles in transformation and recombination (Kupfer and McCarthy, 1992). The *rec2* gene is required for, and induced during, competence development (Gwinn et al., 1997). Also associated with competence development are genes that encode a homolog of *E. coli* single-stranded DNA binding (SSB) protein, RadC and a periplasmic ATP-dependent ligase (Redfield et al., 2005). Ligases are enzymes that seal nicks in DNA and are involved in replication, recombination and repair. The SSB protein has essential roles in DNA replication, recombination and DNA repair (Raghunathan et al., 2000). RecC is required for growth-medium-dependent repair of DNA strand breaks, and functions specifically in recombinational repair that is associated with the replication fork (Saveson and Lovett, 1999).

Noncapsulate *H. influenzae* is frequently found in the airways of patients with chronic obstructive pulmonary disease (COPD). Neutrophils are also found in large numbers in sputum from patients with COPD. *H. influenzae* are phagocytosed by neutrophils, thereby activating a respiratory burst (Naylor et al., 2007). However, instead of killing the bacteria, the neutrophils themselves are killed. Mutants defective in the *rec1* gene (the *recA* homolog) are very sensitive to killing by H_2O_2, suggesting that Rec1 plays a role in survival under conditions of oxidative stress (Sanchez-Rincon and Cabrera-Juarez, 1991). Overall,

these findings suggest that *H. influenzae* may protect its genome against the ROS produced by the host's phagocytic cells by recombinational repair during transformation (sex).

3.5. S. pneumoniae

S. pneumoniae is an important opportunistic human pathogen. It resides asymptomatically in the nasopharynx of healthy carriers, but in susceptible individuals, such as children, elderly and immunocompromised people, the pathogen can spread to other organs and cause disease. *S. pneumoniae* is the principal cause of community-acquired pneumonia and meningitis in children and the elderly, and of septicaemia in HIV-infected persons.

In *S. pneumoniae*, competence to undergo transformation is induced by DNA damaging agents, i.e., the fluoroquinolone topoisomerase inhibitors (norfloxacin, levoflozacin and moxifloxacin) (Prudhomme et al., 2006). Claverys et al. (2006) suggested that these findings are most consistent with a direct contribution of genetic transformation to repair and/or recombination. *S. pneumoniae* stimulates polymorphonuclear leukocytes to produce an oxidative burst (Kragsbjerg and Fredlund, 2001). The *htrA* gene, which is required for virulence of *S. pneumoniae*, is also necessary for resistance to oxidative stress, and for efficient transformation (Ibrahim et al., 2004), suggesting a linkage between virulence, capability to undergo genetic transformation and resistance to oxidative stress (including oxidative DNA damage).

At least 23 genes are required for transformation in *S. pneumoniae*, including *recA* (Peterson et al., 2004; Mortier-Barriere et al., 1998). The *recA* gene in *S. pneumoniae* is part of a competence-inducible operon and is essential for both recombination and repair of DNA damages (induced by UV or methylmethane sulfonate) (Martin et al., 1995). Interestingly, the operon also includes the gene for the main autolysin gene in this species, *lytA*; both *recA* and *lytA* are induced by the quorum-sensing factor (a released peptide hormone that accumulates at high cell densities), and the released DNA is picked up by the surviving cells (Alloing et al., 1998; Mortier-Barriere et al., 1998; Lewis, 2000).

S. pneumoniae exhibits two contrasting life-styles, a planktonic and sessile life-style (Oggioni et al., 2006). The sessile life-style is associated with a pattern of expression similar to that in a pneumococcal model of biofilm. The genes specifically expressed in this sessile/biofilm form include oxidative stress and competence genes. The biofilm model *in vitro* depends on addition of synthetic competence stimulating peptide (CSP), and a biofilm is not formed by CSP receptor mutants. The sessile form is associated with infection of tissues such as brain and lung. Induction of the competence system by the quorum-sensing peptide CSP, not only induced biofilm formation but increased virulence in pneumonia. When *S. pneumoniae* in the different physiological states is used directly to infect mice, sessile cells grown in a biofilm are more effective in inducing meningitis and pneumonia, while planktonic cells from liquid culture are more effective in inducing sepsis. Oggioni et al. (2006) also discussed the use of chemical derivatives of the quorum-sensing molecules to target quorum-sensing regulatory mechanisms as a novel approach to anti-bacterial drug development. Overall, the evidence indicates that in *S. pneumoniae* competence is induced by DNA damaging agents and is associated with increased resistance to oxidative stress and increased RecA, as might be expected if transformation is an adaptation for repairing oxidative DNA damages. Induction of competence also increases virulence leading to meningitis and pneumonia. This suggests that ability to fend off the host's defenses by repairing DNA damages, such as those resulting from the oxidative burst produced by polymorphonuclear leukocytes, may contribute to virulence. The induction of the biofilm form by CSP suggests that sex (transformation) is favored by high density and close proximity.

3.6. S. mutans

S. mutans, the primary etiological agent of human dental carries, lives in biofilms on the tooth surface. Wen et al. (2005) presented evidence that a gene product, designated RopA, is a key regulator of acid and oxidative stress tolerance, genetic competence, and biofilm formation, all critical virulence properties of *S. mutans*. A peptide pheromone quorum-sensing signaling system controls genetic competence in *S. mutans* (Li et al., 2001). The system functions optimally when the cells are living in actively growing biofilms. Biofilm-grown *S. mutans* cells were transformed at a rate 10- to 600-fold higher than planktonic *S. mutans* cells. These results suggest that facultative sex in *S. mutans* is favored under conditions of stress and/or the high cell density characteristic of biofilms where there is maximal opportunity for interaction between the competent cell and the DNA from nearby lysed cells.

3.7. tH. pylori

H. pylori causes chronic gastritis, peptic ulcers and has been implicated in gastric cancer and gastric mucosa-associated lymphoid tissue lymphoma. *H. pylori* is naturally competent for transformation by a process which involves the recombinational repair protein RecA (Fischer and Haas, 2004). The pathogenesis of *H. pylori* depends on its survival in a harsh environment that includes phagocytes and their released ROS (Olczak et al., 2002). *H. pylori* mutants defective in *ruvC* Holliday junction resolvase, an essential protein for recombinational repair, showed increased sensitivity to DNA damaging agents (Loughlin et al., 2003). In addition, *ruvC* mutants were sensitive to oxidative stress, exhibited reduced survival within macrophages and were unable to establish successful infection in a mouse model. These findings suggest that transformation-mediated recombinational repair assists in overcoming oxidative DNA damage in *H. pylori* and contributes to successful infection.

3.8. Other proposed benefits for sex in bacteria

In our discussions, we argued that recombinational repair is the main short-term benefit of transformation in bacteria. The

robustness of recombinational repair is illustrated by reassembly of shattered chromosomes in the bacterium *Deinococcus radiodurans*. This organism, which contains two copies of the genome, can survive extremely high exposures to desiccation and ionizing radiation, which shatter its genomes into hundreds of short random DNA fragments. The mechanism by which the genome is accurately restored, although not a mating process, involves both *recA*-mediated homologous recombination and a process referred to as extended synthesis-dependent strand annealing (Zahradka et al., 2006). However, other adaptive roles for the benefit of transformation and sex in bacteria have been proposed, which we now consider.

3.8.1. Transmission of antibiotic resistance by transformation

The spread of antibiotic resistance genes between bacteria occurs most often by the gene transfer process of plasmid-mediated conjugation and sometimes by phage-mediated transduction. As discussed above, the former process is controlled by plasmid genes and the latter process appears to reflect an infrequent mistake in the assembly of the phage particle. Thus, these processes are not ordinarily regarded as bacterial sexual processes. The bacterial sexual process of transformation, however, can occasionally allow natural transfer of antibiotic resistance plasmids. For example, natural plasmid transformation occurs, albeit infrequently, in *E. coli* (Tsen et al., 2002). Some bacteria appear to have acquired antibiotic resistance by transformation from other bacteria, often from members of a different species. Examples include penicillin-resistance in *S. pneumoniae*, *N. gonorrhoeae*, and *N. meningitidis* as well as sulfonamide resistance in *N. meningitides* (Maiden, 1998; Maynard Smith et al., 1991, 1993). These resistant strains express variants of the antibiotic target (e.g., penicillin-binding proteins) that are metabolically active but exhibit a lowered affinity for the antibiotic. The genes encoding these resistant proteins appear to be mosaics comprising genetic information derived from the host bacterium and other bacteria. These mosaics are thought to arise by occasional intragenic recombination events resulting from transformation. Interspecies chromosomal transformation of rifampicin and erythromycin resistance genes from various *Bacillus* species to *B. subtilis* has been reported (Harford and Mergeay, 1973). Interspecies chromosomal transformation of streptomycin resistance genes to *H. influenzae* from different *Haemophilus* species has also been reported (Albritton et al., 1984). Majewski and Cohan (1999) showed that successful transfer of heterologous genes occurs by homology-facilitated illegitimate recombination, or HFIR. In HFIR, a heterologous sequence that is flanked by homologous DNA can be incorporated into the recipient, because in some recombination systems (maybe all) sequence identity between donor and recipient is required only at the ends. Majewski and Cohan (1999) suggested that HFIR may help explain rapid spread of novel adaptations, such as antibiotic resistance genes, among related species.

3.8.2. The variation hypothesis

In a comprehensive review of bacterial transformation, Dubnau (1999) discussed the hypothesis that bacterial transformation is a mechanism for exploring the fitness landscape. He noted that although all genetic diversity ultimately derives from mutation, recombination can also generate new allelic combinations. He further pointed out that there are many examples of gene transfer in bacteria in which transformation likely played a role, and discussed a case of natural genetic exchange between transformable *Hemophilus* and *Neisseria* species. Although transfers of DNA between these species do not occur often, clear examples of transfer from *Haemophilus* to *Neisseria* of the *sodC* gene and *bio* gene cluster were detected (Kroll et al., 1998). As discussed above, interspecies genetic transfer can occasionally give rise to antibiotic resistance. Dubnau (1999) also discussed evidence that naturally transformable *H. pylori* is genetically one of the most diverse bacterial species so far reported, and is also subject to the highest known rate of intraspecific recombination (Suerbaum et al., 1998). Genetic exchange in *H. pylori* is so frequent over the long-term that different loci and polymorphisms within each locus are all at linkage equilibrium. However, analysis of *H. pylori* strains from different infected individuals in the same family suggested that over short time periods *H. pylori* displays clonal descent. Frequent recombination will tend to rapidly disperse an advantageous mutation to many genetic backgrounds, preventing bottleneck effects (e.g., caused by selective sweeps) from reducing diversity. Taken together, these examples indicate that bacterial transformation can infrequently introduce new advantageous genetic information from other species and may help maintain genetic diversity within a species. Thus, a possible advantage of transformational recombination in bacteria is the uptake of new genes from another bacterial species facilitated by illegitimate recombination (Cohan and Perry, 2007; Cohan, 2002). Uptake of new genes has been suggested as the most likely mechanism of invasion of new ecological niches in bacteria (Gogarten et al., 2002). If the environment is changing rapidly with a high turnover of species (i.e., high speciation and extinction rates, the "speedy speciation" model of Cohan and Perry (2007), then the ability to take up new genes by transformational recombination may be the only way a bacterial lineage could survive for any length of time.

Mortier-Barriere et al. (1998) also hypothesized that transformational recombination evolved as a means of acquiring new genetic traits. They suggested that uptake of DNA from related species present in the same ecological niche followed by recombination into the chromosome can provide the recipient genome with enhanced plasticity. On the same lines, Prudhomme et al. (2006) and Claverys et al. (2006) proposed that transformation in *S. pneumoniae* may play a crucial role in generating genetic diversity under stress conditions, implying that some of the variants produced might be better able to survive the stress. Although these effects of competence and transformation on genetic variation can be beneficial to the pathogen (mainly in the long-term), we know of no evidence supporting an immediate, consistent large

benefit of recombinational variation comparable to the likely benefit of recombinational repair in removing DNA damages. Indeed, in most occurrences of transformation the DNA received by the recipient is likely from a nearby clonally related donor, so that no genetic variation should be produced. We consider that transformation is maintained in bacteria primarily by the immediate, short-term, adaptive value of recombinational repair, but recombinational variation, occasionally produced as a byproduct, can have substantial evolutionary consequences over the long-term.

3.8.3. Competence-for-feeding hypothesis

A rather different view on the short-term adaptive role of transformation was put forward by Redfield (2001, 1993b), who proposed that competence evolved to permit the uptake of DNA as a food supply. This proposal was made, in part, because of perceived inadequacies in both the variation hypothesis and the DNA repair hypothesis. With respect to the variation hypothesis, she reasoned that the success of genes causing transformation is limited by several factors, but especially the poor quality of DNA derived from dead cells (Redfield, 1988, 1993b; Redfield et al., 1997). With respect to the repair hypotheses, she performed experiments showing that in both *H. influenzae*. and *B. subtilis* competence is not induced by two DNA damaging agents, mitomycin C and UV (Redfield, 1993a). She thus concluded that these results are inconsistent with the DNA repair hypothesis. However, Dubnau (1999) pointed out that induction of competence directly by DNA damaging agents is not a strong prediction of the repair hypothesis. According to Dubnau (1999) induction of a DNA repair system before the appearance of DNA damage would be selectively advantageous. Such an induction mechanism would respond to conditions in which DNA damage is likely to occur, but not to the damage itself. For instance, as *B. subtilis* approaches stationary phase, increased stress may trigger a multi-faceted response that includes induction of competence. Thus, induction of competence in some organisms may be a component of a broader response to stresses that ordinarily cause DNA damage. However, as discussed above, competence in *S. pneumoniae* was recently found to be induced by DNA damaging agents (topoismerase inhibitors and mitomycin C) (Prudhomme et al., 2006). Thus, in some organisms DNA damage may directly induce competence. Additional counter-arguments to Redfield's conclusion were presented by Michod and Wojciechowski (1994).

Further support for the competence-for-feeding hypothesis comes from experiments with *H. influenzae* showing that supplementation of starvation media (in which competence is ordinarily induced) with physiological levels of AMP and GMP substantially reduced competence induction (MacFadyen et al., 2001). This finding was interpreted to indicate that depleted purine pools signal the need for nucleotides, and to support the hypothesis that competence evolved primarily for nucleotide acquisition. However, since we have known for some time that competence is induced by starvation, the reduction in competence by added AMP and GMP could have been due, at least in part, to the alleviation of starvation induced stress and the damages it produces. Thus, these experiments are consistent with the idea that nutrient-deprivation causes competence via a stress-induced signaling pathway. Induction of competence by stress in bacteria may occur in anticipation of stress-induced DNA damage and may be analogous to the induction of sex during nutrient-deprivation in facultatively sexual eukaryotes where gamete recognition and fusion ensures the availability of DNA template for the recombinational repair of such damages. These considerations can help explain the association of competence with recombination, something a pure nutrition based hypothesis cannot do.

To argue for this scenario is the fact that competent *B. subtilis* cells maximally express both damage inducible SOS-like genes (Yasbin, 1977; Love et al., 1985) and the recombination system (Lovett et al., 1989). Why would competent cells do this if they were not using the transforming DNA as template for DNA repair? Noteworthy, in all of our experiments with *B. subtilis* discussed earlier, cultures were grown in conditions promoting maximal competence. Nevertheless, the homologous transformation rate responded adaptively and increased in response to increased DNA damage.

Nutrient limitation could mean a shortage of nucleotides in the cell, but then why would competent *B. subtilis* cells also export DNA (Streips and Young, 1974; Crabb et al., 1977; Sinha and Iyer, 1971; Lorenz and Wackernagel, 1994)? In addition to *B. subtilis*, *N. gonorrhoeae* also secretes chromosomal DNA via a complex secretion system, and this DNA may be used in natural transformation (Hamilton et al., 2005). The finding, discussed above, of dynamic RecA/ssDNA nucleofilaments that may scan the recipient chromosome for regions of homology (Kidane and Graumann, 2005) also seems incompatible with the concept that transforming DNA is taken in mainly as food.

Dubnau (1999) concluded that uptake of DNA as a food supply is not likely to be a major factor among the selective pressures that maintain the competence mechanism for the following reasons: (1) *B. subtilis* possesses a powerful nonspecific nuclease that is secreted into the medium, as well as uptake systems for the nucleolytic products. This would presumably provide an efficient route for the consumption of environmental nucleic acids. Why would an elaborate transformation machinery evolve to meet this need, when it could be met by a simpler and more generally useful pathway? (2) Because one strand equivalent is released into the medium, the competence machinery discards half of the potential food, which is a wasteful mechanism. (3) *H. influenzae* and *N. gonorrhoeae* systems exhibit uptake specificity. This does not suggest a food-gathering mechanism. Hamilton et al. (2005) also noted that uptake of specific DNA is more consistent with a role for transformation in genetic variation or DNA repair rather than in acquisition of nutrients. Likewise Claverys et al. (2006) pointed out that the induction of competence by DNA damaging agents and antibiotics in *S. pneumoniae* is difficult to reconcile with the competence-for-feeding hypothesis.

3.8.4. Parasitic DNA hypothesis

Hickey (1982, 1993) proposed that sex arose in bacteria as an adaptation of molecular symbionts such as transposons and plasmids to promote their own spread. He also suggested that this symbiont theory has implications for subsequent stages in the evolution of sex. Thus, according to this theory sex arose and is at least partly maintained as an unavoidable imposition by parasitic or mutualistic genetic elements. In other words, there is no adaptive value to sex for the host of these elements. This explanation likely applies to the origin of conjugation and transduction, since these processes are mediated by plasmid or phage genes. However, as discussed by Bernstein et al. (1987), this explanation seems implausible to us as a general explanation for transformation, because it implies that sex is unavoidably maintained despite an excess of costs over benefits.

4. Fungal pathogens

4.1. Overview

In addition to the prokaryotic pathogens described above, possible sexual reproduction has also been described in several eukaryotic microbial pathogens, including fungal pathogens (infecting humans: *Cryptococcus neoformans*, *Candida albicans*, and *Aspergillus fumigates*, or infecting plants: *Ustilago maydis*), the oomycete plant pathogen *Phytophthora infestans*, as well as several human protozoan parasites, such as *Toxoplasma gondii*, *Trypanosoma brucei*, *Trypanosma cruzi and Plasmodiium falciparum* (reviewed in Heitman, 2006). To date, with the exception of the two fungal pathogens discussed next, there is insufficient data to evaluate the adaptive value of sex in these organisms.

4.2. C. neoformans

C. neoformans is a basidiomycetous fungus that is a common opportunistic pathogen of immunocompromised hosts, particularly AIDS patients. It is spread by aerosolized spores and upon infection disseminates to the central nervous system where it can cause meningoencephalitis. Notably, *C. neoformans* survives phagocytosis by macrophages and proliferates within these cells (Fan et al., 2005). Intracellular survival may be the basis for latency, disseminated disease, and resistance to eradication by antifungal agents. The mechanism by which it survives the hostile intracellular environment of the macrophage involves up-regulation of genes involved in responses to oxidative stress.

The vast majority of environmental and clinical isolates are mating type α. Haploid filaments of mating type α can undergo a process of diploidization (perhaps by endoduplication or stimulated cell fusion) to form cells called blastospores. The diploid nuclei of blastospores undergo meiosis, including recombination, to form haploid basidiospores that can then undergo dispersal (Lin et al., 2005). This process is called monokaryotic fruiting. Required for this process is *dmc1*, a conserved homologue of genes that mediate DNA double-strand-break repair and homologous chromosome pairing during meiosis in yeasts and mammals. The benefit of meiosis to the fungus was suggested to include promotion of repair in a DNA damaging environment, including the host. Thus, *C. neoformans* can undergo a meiotic process, monocaryotic fruiting, that may promote recombinational repair in the oxidative environment of the host, and this may contribute to its virulence. This process does not fit our definition of sex because there is no outcrossing between different individuals, but we have discussed it here because it illuminates the function of meiotic recombination in a pathogen. Recombinational variation is not likely to be a sufficient benefit to explain the costs of monokaryotic fruiting in *C. neoformans*, because the diploid nuclei that undergo meiosis are formed from clonally related haploid nuclei.

4.3. U. maydis

U. maydis is a pathogenic basidiomycete fungus that infects maize, one of the world's major cereal crops, resulting in severe economic losses. It is a model organism for the study of plant–microbe interactions, and has a well-established life-cycle (Steinberg, 2007). The infection of maize by *U. maydis* requires haploid, budding yeast-like cells of compatible mating types to fuse (a pheromone mediated mating process), and grow as dikaryotic, filamentous cells which penetrate and colonize plant tissue. Infection results in the formation of plant tumors at sites of infection. Within these tumors, the dikaryon proliferates. Nuclear fusion then occurs, and diploid cells are generated which differentiate into melanized diploid spores (teliospores) that eventually rupture the plant's cells causing the tumors to disperse. The teliospores undergo meiosis and produce meiotic haploid progeny capable of reinitiating the life-cycle.

Plants have developed efficient defense systems against pathogenic microbes. One of the most rapid plant defense reactions after pathogen attack is the oxidative burst, which involves the production of ROS at the site of the attempted invasion. *U. maydis* has an oxidative stress response, regulated by a gene *yap1*, which protects the pathogen from this attack, and is necessary for virulence (Molina and Kahmann, 2007). In addition, *U. maydis* has a well-established recombinational repair system (Kojic et al., 2006). This system involves a Rad51 homolog that is very similar in sequence and size to the mammalian counterparts, a protein, Rec2 that is more distantly related to Rad51, and the Brh2 protein that is a streamlined version of the mammalian Breast Cancer 2 (BRCA2) protein. Inactivation of any of these proteins causes increased sensitivity to DNA damaging agents, deficiency in mitotic recombination, increased mutation, and failure to complete meiosis. These findings suggest that recombinational repair during meiosis in *U. maydis* may assist the pathogen in surviving DNA damage arising from the host's oxidative defensive response to infection.

5. Discussion

5.1. Areas of general agreement and disagreement

Although sex is ubiquitous in nature and is frequent even in bacteria and viruses, there is, as yet, no general agreement on

the fundamental benefit of sex. Any general explanation for the adaptive value of sex must provide a sufficient advantage to balance the costs of sex that are often large. There is broad agreement that understanding the adaptive function of recombination during sex is the key to understanding the adaptive value of sex. Also there is good evidence that the proteins involved in carrying out recombination in eukaryotes evolved from proteins with similar functions in prokaryotes, suggesting continuity of the function of the recombination machinery. There also appears to be general acceptance of the idea that recombination serves a repair function during vegetative growth of viruses, bacteria and during mitotic divisions of somatic cells of multicellular organisms. However, when it comes to the recombination events during sexual processes (e.g., viral mixed infection, bacterial transformation, and meiotic recombination), evolutionary biologists often assume that the repair function of the recombination machinery is superseded by the function of generating variation so as to reduce genetic associations among alleles that interfere with selection.

5.2. The balance between the costs and benefits of sex in pathogens

5.2.1. Costs of facultative sex in viruses, bacteria and fungi

The cost of mating appears to be substantial in facultative sexuals. In viruses, sex in the form of multiple infection, has costs. For several types of virus, when a single virus infects a cell its genome quickly expresses gene products which exclude additional viruses from infecting the same cell. Presumably this is an adaptation for preserving the infected cell as an exclusive resource for the first virus to enter. This then allows the reproduction of the virus. As an example, when an undamaged phage T4 injects its DNA into a host cell, it establishes a barrier to infection by a second phage T4 within 2 min. However, if the first infecting phage is treated with UV, its barrier to infection by a secondary phage is reduced (Bernstein, 1987). Thus, damage to a first-infecting phage shifts its life-cycle from a strictly multiplicative mode towards a sexual (recombinogenic) mode, as would be expected if MR were an adaptation for repair. However, the price paid is the loss of exclusive use of the host cell. Indeed, Drake (1958) showed that an infecting poliovirus is capable of interfering with multiplication of a secondary infecting poliovirus, but polioviruses inactivated by UV lose their interfering ability. This can then lead to MR, but again at the price of losing exclusive use of the host cell as a resource for its replication.

In bacteria capable of transformation, a major cost of sex is the cost of competence and DNA uptake, which involve the expression and activity of the numerous genes. Transformation in bacterial species is often promoted by the production of an extra-cellular factor that, upon release into the surrounding medium, induces the competent state in neighboring cells in the population. Competence pheromones have been described in *B. subtilis* (Charpak and Dedonder, 1965; Pariiskaya and Pukhova, 1967; Solomon and Grossman, 1996) and *S. pneumoniae* (Havarstein et al., 1995). Other processes required for transformation are also costly. For instance, the integrated action of multiple gene products is required for the binding of DNA to the recipient cell surface followed by its uptake into the cell through the outer membrane (in gram negative bacteria) and then through the cell wall and cytoplasmic membrane (Dubnau, 1999; Chen and Dubnau, 2004). These specialized machines to transport DNA into the cytoplasm appear to be energy requiring. In *B. subtilis* the proton motive force functions as a driving force for DNA uptake (van Nieuwenhoven et al., 1982).

A cost of mating has been demonstrated in the pathogenic facultative sexual fungus, *C. neoformans*, discussed earlier (Xu, 2005). It was estimated that sexual interaction between *a* and α mating types exacts about a 10% reduction in vegetative fitness as measured by generation time. This cost has two components, the cost of producing mating signals that exert effects on mating partners, and the costs associated with responding to active mating partners.

Mating signals have also been studied in *U. maydis*, discussed above. Here, mating is linked to the pathogenic process and is initiated on the host plant surface when a yeast-like *U. maydis* cell recognizes a compatible pheromone secreted by a partner cell. Pheromone perception involves specific receptors and triggers a morphogenic yeast-hypha transition, leading to hyphal fusion (mating), dikaryon formation, invasion of the host cell, nuclear fusion and meiosis (Steinberg, 2007; Spellig et al., 1994).

5.2.2. The benefits of sex in viruses, bacteria and fungi

According to the repair hypothesis, the short-term benefits of sex are to be found in the removal of potentially lethal damage in the genomes passed on to the next generation. This conclusion is in accord with that of Cox (1991, 1993, 2001) who analyzed the experimental evidence on the molecular interactions of the *E. coli* RecA protein with DNA. Homologs of the *recA* gene are widespread in nature and play a central role in sexual recombination in bacteria and eukaryotes. Cox reasoned on mechanistic grounds that the RecA protein evolved as the central component of a recombinational repair system, and that the generation of genetic diversity is sometimes a useful byproduct. Cox's conclusion implies that sex, itself, is primarily an adaptation for DNA repair. We consider that the repair hypothesis, in contrast to the variation hypothesis, provides an appropriate explanation for the adaptive advantage of sex in the short-term, since its benefits are large enough (removal of potentially lethal damages) to plausibly balance the large costs of sex, and it can be consistently applied to all organisms that have sex, including the facultative sexual microorganisms discussed in this paper. This is not to deny that in the long-term the variation produced by sex may serve to increase the rate of adaptation (Goddard et al., 2005; Colegrave et al., 2002; Kaltz and Bell, 2002; Cooper et al., 2005; de Visser and Elena, 2007; Peters and Otto, 2003). As mentioned earlier, a trait like sex which is so embedded in the biology of life is bound to have a variety of significant effects.

As discussed in the Introduction, a popular elaboration of the Red Queen hypothesis proposes that rapid genetic change in

parasites and pathogens selects for sex in the host. The key requirement for the Red Queen hypothesis to be met in nature is that there be strong selection per gene mediating the species interaction, such that combinations of alleles switch from being advantageous to disadvantageous and back again over the course of a few generations (Hamilton, 1980; Seger and Hamilton, 1988). However, mathematical analysis of this hypothesis has made it seem doubtful that strong selection per gene is sufficiently commonplace for the Red Queen hypothesis to explain the ubiquity of sex (Barton and Charlesworth, 1998; Otto and Nuismer, 2004; Otto and Gerstein, 2006; Gandon and Otto, 2007). Furthermore, negligible evidence from the medical literature on infectious disease has been put forth to provide support for this theory. The situation is similar for plant pathogens. The main concept on the genetic basis for disease resistance in plants differs from the Red Queen hypothesis, and is known as gene-for-gene resistance, a concept widely exploited by plant breeders, forming a cornerstone of disease control in crop plants (Yu et al., 1998; Kaloshian, 2004). The designation ''gene-for-gene'' denotes the dependence of disease resistance on matched specificity between a plant disease resistance gene and a pathogen avirulence gene. Parker (1994), after reviewing numerous genetic studies on plant disease resistance, failed to uncover a single example consistent with the concept that pathogens are the primary selective agent responsible for sexual reproduction in their hosts.

6. Conclusions

The recombinational component of sex is the most ancient aspect of sex. As reviewed above, microbes undergo several processes that involve recombination; thus, understanding the adaptive role of recombination in these lineages will provide clues as to the ancestral primary function of sex. We argued here that in prokaryotes, the DNA repair is likely most basic aspect of recombination as this benefit does not require that the incorporated DNA carry different alleles (although it does not preclude this). While creating genetic variation is fundamental for the evolutionary process, there are several ways variation can be achieved in the absence of recombination, including the recent finding that coevolution with viruses might increase mutation rates in bacteria and confer the benefits of genetic variation in the absence of sex (Pal et al., 2007). On the other hand, for haploid lineages—such as the prokaryotes, the efficient repair of DSB damages that occurred before DNA replication is dependent on sex and recombination. As the prokaryotic cell organization is the ancestral type of cellular life, we believe that the repair of DNA was likely the ancestral adaptive role of recombination and of sex. This is also supported by considerable evidence indicating that the molecular machinery that carries out sexual recombination is adapted for repair.

The studies on sex in pathogenic bacteria, viruses and fungi reviewed above are consistent with the idea that sex provides an immediate benefit by allowing repair of genome damage, particularly damages that occur in the inflammatory, oxidizing environment associated with infection. Viral, bacterial and fungal pathogens have in common that to be successful they need to survive the defense systems of their host. As reviewed above, these systems often involve ROS. The genome of these pathogens is a vulnerable target as it is well known that ROS damage DNA (Slupphaug et al., 2003). In humans, phagocytic cells engulf invading pathogenic microorganisms and employ a respiratory burst, involving the release of oxidative free radicals, to destroy the invaders. The pathogenicity of such microorganisms may depend to a large extent on their ability to defend themselves against oxidative damage and its consequences, including damages to their genetic material. Sex leading to recombinational repair is an effective means of dealing with such damages. Thus, sexual processes in pathogenic bacteria and viruses appear to be relevant to the survival and spread of infectious disease.

Recombination during sexual processes in viruses and bacteria is also a source of variation; but in the short-term this is probably not beneficial since most new non-neutral recombinational variants, like most new non-neutral mutations, are deleterious as they break up gene combinations that work. However, over the long-term infrequent recombinational variants may arise which aid the spread of infection and are thus beneficial to the species. These long-term benefits may have some role in maintaining sex, but we think, not the principal role because of their low incidence. Rather, we consider that the immediate benefit of resisting oxidative attack by the host is likely the principal selective force maintaining sex in these organisms.

The benefit provided by recombinational repair in coping with stress-induced DNA damage can be generalized to all organisms that have sex, even where the source of stress and damage is less obvious. In non-pathogenic facultatively sexual organisms, other stresses such as desiccation, starvation for particular nutrients, or high temperature can cause ROS and genome damage, and provide the selective force maintaining sex. And in obligate sexual organisms there are consistent endogenous sources of stress, such as the ROS produced as byproducts of respiration. Since the average number of double-strand breaks occurring at each cell generation in humans is about 50 (Vilenchik and Knudson, 2003), we think that meiotic recombinational repair is maintained to protect germ cell DNA from the lethal consequences of such damages.

Like with other evolutionary innovations initially driven by one primary factor (see discussion of the origin of insect wings by Lenski, 1999), sex and recombination acquired additional adaptive roles, as sex and recombination had to be re-aligned with other organismal traits emerging throughout the history of life—especially during major evolutionary transitions such as the evolution of the eukaryotic cell, the switch from a predominantly haploid to a predominantly diploid life-cycle, and the transition to multicellularity. Furthermore, depending on the specific developmental, life-history traits, and environmental circumstances by which different lineages are characterized, the relative significance of these benefits may vary. Nevertheless, we propose that in potentially damaging environments—such as those in which microbial pathogen thrive, the benefit of DNA repair is the main selective force driving the maintenance of sex and recombination.

Although, this review is concerned with microbial pathogens, we consider the repair hypothesis to be generally applicable to the evolution of sex in all organisms. Indeed, the need for effective and efficient repair of DNA damage is a universal feature of all of life. A central point of our hypothesis is that the recombinational aspects of sex are primary to life and reproduction, that they evolved early in the history of life (Michod, 1998; Long and Michod, 1995; Michod and Long, 1995; Bernstein et al., 1984; Margulis and Sagan, 1986) and since have had a continuous evolutionary history. There are other fundamental aspects of reproduction in addition to recombination, of course, for example, the mating system, the alternation of haploid and diploid generations, and the timing of recombinational repair and meiosis with regards to other aspects of the reproductive system such as mating and fusion. We do not believe that the need for recombinational repair of DNA damages explains all aspects of the reproductive system in all organisms. However, we do believe that a complete understanding of the reproductive system must involve the need for effective and efficient recombinational repair of DNA damages.

Acknowledgments

We thank Carol Bernstein, Denis Roze, Matt Herron and Deborah Shelton for their critical reviews and helpful comments on earlier versions of the manuscript.

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