

EVOLUTION OF SEX IN PROKARYOTES¹

Richard E. Michod, M. F. Wojciechowski, and Mary Hoelzer

Department of Ecology and Evolutionary Biology
University of Arizona, Tucson, AZ 85721

ABSTRACT Mathematical models and experimental tests of the hypothesis that sex arose early in the history of life for the purpose of DNA repair are summarized. Mathematical models show that it is possible for recovery from genetic damage to be the primary selective force molding the early stages of evolution of the sexual cycle. Natural genetic transformation in bacteria provides a test case for this theory. In the bacterium *Bacillus subtilis*, experimental results show that competent (sexual) wild-type cells survive UV radiation damage better than noncompetent cells if they are allowed to undergo transformation with homologous chromosomal DNA after the UV treatment. The advantage of competent cells goes away if the cells are transformed before UV treatment. These results persist in various strains that are deficient in either excision repair or SOS-like system repair. However, the advantage of competent cells goes away if the competent cells are transformed with non-homologous plasmid DNA. These results support the hypothesis that transformation evolved for the function of bringing DNA into the cell for use as template in recombinational repair.

INTRODUCTION

One of the most obvious consequences of sex in metazoans is that sex between aging adults produces babies that are young. This obvious association led to the view, common in the last century, that sex functioned to rejuvenate life. It was the eminent German biologist August Weismann (1), who, in 1889 first argued against this view stating that "twice nothing cannot make one." Instead, he offered, what he admitted at first sight was a "preposterous" idea, the idea that the function of sex was to produce variability to fuel adaptive evolution. The reason for his

¹This work was supported by NIH grants RO1 GM36410 and KO4 HD00583

apologizing in advance for this idea was that, at the time, it was appreciated that sex undoes what it creates. The very sexual process that creates some new, possibly adaptive, combination of genes, one generation, breaks this combination of genes apart the next generation. However, in spite of this problem, the view that sex evolved as a system for producing genetic variability has prospered. The variation view is now the dominant view, even though it has little experimental or theoretical support. One can appreciate why, at the time of Weismann, the rejuvenation view fell into disfavor, since it lacked an explicit mechanism. However, from our modern vantage point, armed with our understanding of genetic complementarity, redundancy and DNA repair, one can see how two damaged DNA molecules can through recombination make each other whole again. Twice nothing can make one.

At the molecular level, sex can be defined as involving two basic components, (i) **recombination**, in the sense of the physical breakage and reunion of homologous DNA molecules, and (ii) **outcrossing**, in the sense that the homologous DNA molecules that participate in recombination come from two different individuals. The repair hypothesis (ref 2; for review see ref. 3) argues that sex arose in simple haploid organisms, and is maintained in its present forms, for the purpose of providing redundant genetic information for the recombinational repair of damage to the genome.

MODELS FOR THE ORIGIN OF SEX

In this section, we summarize mathematical models of the origin of sex (2,5) that motivate the experimental work on transformation discussed in the next section. To properly phrase the question of the origin of sex, it is necessary to begin with some assumptions concerning the primordial genome. We believe that these assumptions are generic, in the sense that they apply to any primordial genetic material, based on the properties that it must have, independent of the precise chemistry involved. We adopt the Eigen view that the hypercycle, a set of mutually independent interacting units, is an appropriate model for the primordial genome. The characteristics of hypercycles have been extensively studied by Eigen and coworkers (4). The basic property of hypercycles used here is that hypercycles can stably maintain their component genes, so long as there is at least one copy of each gene present. The primordial genome is assumed to be segmented, that is, the different genes are assumed to be on separate fragments. There are assumed to be no recombination enzymes at this early stage of evolution of the genetic system.

At some point the primordial genome became encapsulated into a protocell. The precise details of how this came about are highly speculative (2,5), however, once this occurred the problems faced by the early protocell seem obvious, and again appear to be generic. The basic prob-

lem faced by the early protocells are the production of cells that are deficient in one or more genes. The causes of gene deficient cells are varied, but two basic causes are genetic damage and failure of assortment of genes from the parent cell into the daughter cell. Strategies for coping with gene deficient cells most probably involved redundancy, in one form or another.

Intra-cellular redundancy, or multiple copies of each gene in the protocell, allows for recovery from genetic damage, so long as all copies of a particular gene are not damaged at the same time. Our hypothesis that genetic recovery based on redundancy evolved early in the history of life is supported by the phenomenon of multiplicity reactivation in extant single-stranded segmented RNA viruses, e.g. influenza virus, which are among the simplest living systems. In addition to providing benefits as a result of recovery from genetic damage, high redundancy should help solve the assortment problem by increasing the chances that daughter cells have, at the least, one copy of each gene. So long as the protocell has at least one good copy of each gene, additional copies can be generated through the positive feedback inherent in the hypercyclic nature of the interactions between the genes. However, maintaining intra-cellular redundancy has costs, most obviously, the costs of replicating the additional copies of each gene. For simple systems, such as the protocell under consideration, these costs are likely to be a sizeable fraction of the resource, energy and time budget.

Another strategy is for the protocell to remain at low redundancy (say, haploid) for most of the time, but periodically fuse with other cells (to become diploid) for genetic recovery, and then split into two daughter cells (haploid again), so as to reduce the costs of redundancy. This cycling of diploid and haploid states is the sexual cycle in its most basic form, whereas the strategy that maintains multiple copies of each gene without fusion and splitting is the asexual strategy. Recombination, as defined above, refers to the physical breakage and rejoining of DNA molecules. However, during the early stages in the sexual cycle, before genes became physically linked on the same chromosome, recombination in the modern molecular sense did not exist and was probably not necessary. Nevertheless, two of the main effects of recombination in modern systems, genetic variation and genetic recovery (repair), are present in the hypothesized sexual cycle of the primordial protocell.

A number of explicit competition and population genetic models have been constructed embodying the ideas discussed above to determine the conditions under which a sexual strategy would evolve (2,5). The basic assumptions of these models were as follows. (i) Genetically deficient cells were produced both by damage of component genes and by assortment errors at defined rates. (ii) These "gene-dead" cells could be recovered by fusion, if fusion occurred in some specified window of time. (iii) Costs of redundancy were included by specifying that the rate of replication of a protocell was inversely related to its ploidy level

(intra-cellular redundancy). (iv) Benefits of high ploidy (high intra-cellular redundancy) were included by assuming that a cell did not become gene-dead unless all of its copies of a gene were damaged. Furthermore, cells with high ploidy were more likely to successfully assort genes into daughter cells so that daughter cells had at least one copy of each gene. (v) Costs of sex and fusion were also assumed.

The basic conclusion of these models was that the sexual strategy outcompeted the asexual strategy under most conditions except those of low levels of damage. Furthermore, conditions were specified under which a rare sexual mutation could increase in an asexual population as a result of its ability to mate with and to recover gene-dead cells, even though only one-half of its daughter cells contained the gene for sex. Consequently, these models and the ideas they embody provide an explanation for both aspects of the sexual cycle, fusion and splitting. Fusion evolved so as to use redundancy to recover genetic information that was lost through damage or assortment. Splitting evolved so as to reduce the costs of redundancy. These models show that it is possible for recovery from genetic damage to be the primary selective force molding the early stages of evolution of the sexual cycle. In the next section natural genetic transformation in bacteria is studied as a test case for this theory.

EXPERIMENTAL RESULTS ON TRANSFORMATION

Genetic transformation is a highly evolved attribute of cells and occurs naturally in a number of modern bacteria, including *Haemophilus* and *Bacillus* (6). It results from the ability of cells to become "competent" to bind and take up DNA molecules from the environment. The incorporation of this DNA into the bacterial chromosome by the physical recombination of homologous strands is catalyzed by enzymes involved in both recombination and DNA repair. The acquisition of physiological competence for transformation in *B. subtilis*, and in other naturally competent bacteria, is a complex, energy-requiring, developmental process and not simply the consequence of passive entry of DNA into a cell (7). Nor is natural transformation in bacteria like *B. subtilis* similar to the artificially inducible uptake and transformation of *Escherichia coli* and other cells. In nature the sources of transforming DNA have not been well defined but there is evidence that *B. subtilis* cells actively export DNA to the environment during growth. In addition, DNA is likely to be released from cells following cell death and lysis (6,7).

In modern sexual systems, outcrossing (or fusion) is eventually followed by reproduction, that is DNA replication and, usually two, cell divisions resulting in haploid gametes. However, the fusion/splitting aspects of the sexual cell cycle, or, in modern form, the alternation of generations, is not a basic aspect of sex in general. This is because it is not logically necessary that sex, as defined above, be coupled with

either DNA replication or reproduction. For example, both recombination and outcrossing are essential components of natural genetic transformation in bacteria, but sex in these cells is not associated with either DNA replication or reproduction of the cell. Indeed, reproduction is strictly asexual or mitotic-like in all sexual prokaryotes, even though the cell may have previously had sex, that is recombined DNA from another individual. There are no costs of maintaining the extra genetic redundancy with transformation, since the additional template brought into the cell is discarded after it is used for repair. Transformation seems to have the best of both worlds, it provides DNA template for repair, while avoiding the costs of replicating extra chromosomes. A transformation-like process may have preceded cell fusion in the early evolution of the sexual cycle.

There are other important differences and similarities between transformation and the modern sexual cycle. The function of competence is similar to that of fusion or mating, that function being to promote outcrossing. As with fusion, there are physiological costs to becoming competent and taking up exogenous DNA. For example, the development of the competent state is characterized by a decrease in nucleic acid synthesis, a latency in cell growth and multiplication, and the synthesis of a set of competence-specific proteins (6,7). In addition, resident prophages are preferentially induced by UV in competent cells leading to a decrease in cell viability and the frequency of transformed cells. For this reason our experiments have been conducted on strains of *B. subtilis* that have been cured of these resident prophages. Unlike sexual systems in more complicated organisms, transformation is not a reciprocal process in terms of the contribution of each "parent," there being a "donor" of genetic information (the transforming DNA) and a recipient (the transformed cell).

Although much is known about the mechanisms of DNA uptake, processing and recombination during transformation in bacteria such as *B. subtilis* (for review see ref. 7), the primary function(s) of transformation and the evolutionary advantages it confers upon cells have received little attention and thus are not well understood. It has often been assumed that transformation evolved for the purpose of genetic variability, or simply as a means for nutrient and/or energy acquisition (6). Recently, we have argued that the evolutionary function of transformation (and presumably competence development itself) in *B. subtilis* lies in its role in providing the cell with homologous DNA molecules for DNA repair (8,9). In this section we summarize this work, as well as present preliminary results from experiments designed to test whether recombination during transformation is targeted to damaged sites in the DNA.

The repair hypothesis (2) argues that genetic transformation in bacteria such as *B. subtilis*, like recombination in eukaryotes, evolved and confers a selective advantage as a system for DNA repair. To test this hypothesis for the function of transformation, our initial approach has

been to study the relative densities (i.e., survival) of transformed (sexual) cells and total (primarily asexual) cells in competent cultures of *B. subtilis* strains as a function of the dose of a potent DNA damaging agent, such as ultraviolet radiation (UV) or hydrogen peroxide. We have done so under conditions in which cells are transformed either with highly purified homologous (i.e., chromosomal) or non-homologous plasmid DNAs before (DNA-UV) or after (UV-DNA) UV irradiation of the cultures (8,9).

The results of these experiments using a *B. subtilis* strain that is wild-type for recombination and repair (*rec*⁺) show there is a qualitative difference in the relationship between the survival of transformed cells and total cells in the UV-DNA and DNA-UV treatments (Fig. 1, ref. 8; Fig. 1a below). In the UV-DNA treatment, cells transformed with homologous DNA (chromosomal markers) had a greater average survivorship than total cells. In DNA-UV treatments this relationship was reversed. Moreover, we find a consistent and qualitative difference between the UV-DNA and DNA-UV treatments in the relationship between the rate of transformation (or transformation frequency) with homologous DNA and UV dosage. The homologous DNA transformation rate increases with UV dose in UV-DNA treatments but decreases with UV dose in the DNA-UV experiments (Fig. 4, ref. 8), relative to the transformation rate in undamaged cells. The transformation rate is defined as the number of single marker transformants divided by the number of total viable cells at a given UV dose, and we calculate for each experiment the ratio of the transformation rate at each UV dose to the transformation rate in unirradiated (0 J/m²) cultures.

We have entertained two hypotheses as likely explanations for our results, both of which are consistent with the predictions of the repair hypothesis (8,9). The survival of competent cells should increase relative to that of noncompetent cells in a UV-DNA experiment, either because (i) UV-induced DNA damage directly increases the frequency or efficiency of transformation, or because (ii) competent cells are more resistant to UV-induced damage as a result of an enhanced capacity for recombinational repair ("transformational repair"), and thus survive better in a damaging environment. The former may occur as a consequence of an increase in the binding, uptake and/or recombination of homologous transforming DNA in damaged cells, or because the UV-induced damages in the DNA helix themselves stimulate recombination directly (are targets for recombination) or indirectly through the subsequent induction of enzymes essential for homologous recombination. It is important to note that in DNA-UV experiments, there can be no benefit or repair advantage derived from the uptake and integration of homologous DNA since this occurs prior to the DNA damaging treatment, nor can there be any "induction" in the levels of competency or transformation in cells.

There are other inducible factors not directly related to the transformation process, such as excision repair or SOS repair, which may in-

fluence the relative survival abilities of competent and noncompetent cells and these factors may contribute to the above-mentioned differences between transformed and nontransformed cells. For example, the SOS-like system for DNA repair and mutagenesis is elicited during competence development in *B. subtilis* cells, independent of any exposure to DNA damaging agents (10).

To examine these alternative hypotheses and the contribution of the confounding factors just mentioned, we have performed experiments similar to those described above in which we have measured rates of homologous DNA-mediated transformation in two repair-deficient mutant strains, and non-homologous plasmid DNA-mediated transformation in the *rec*⁺ strain. We have also employed the use of operon fusions to study damage inducible genes under the regulatory control of the SOS-like system as a means to assay for their expression in both competent and noncompetent cell fractions in response to DNA-damaging treatments (9). These and other results are summarized in Table 1.

Table 1

SUMMARY OF EXPERIMENTS		
Experiment	Factors Addressed	UV-DNA Result
Plasmid DNA	Rec-dependent Induction Transformation Repair	No Increase in Transformation Rate
<i>recA1</i> Strain	Induction of Excision Repair and SOS Repair	Increase in Transformation Rate
<i>uvrA42</i> Strain	Excision Repair	Increase in Transformation Rate
<i>Din</i> Fusion Expression	Induction of SOS System	Same in Competent and Non-Competent Cells
Damaged Donor DNA	Result Incidental Targeted Uptake	Increase in Transformation Rate

Our experiments with the repair-deficient strains (*recA1*, *Rec*⁻ and *uvrA42*, *Uvr*⁻) demonstrate a similar qualitative difference in the homologous DNA transformation rate between UV-DNA and DNA-UV treatments as seen with the *rec*⁺ strain (Fig. 3-5, ref. 9). This indicates that the general qualitative result that the homologous transformation rate increases with UV dosage in UV-DNA experiments does not depend upon the induction of the SOS-like response or excision repair (Table 1). Moreover, the observed increase in the transformation rate with UV cannot be explained just by a differential induction of SOS-like system

and its associated repair in competent cells, since SOS-like repair functions (*din* operons) are induced in noncompetent cells in response to DNA damage to a similar level (Table 5, ref. 9).

The apparently general result that transformation rates increase with UV in UV-DNA experiments, goes away if *rec*⁺ cells are transformed with non-homologous plasmid DNA, even though in DNA-UV experiments plasmid transformation rates behave in a manner similar to that observed when cells were transformed with homologous chromosomal DNA in DNA-UV experiments (Fig. 2, ref. 9). This result is consistent with there being a repair advantage to homologous transforming DNA, and strongly suggests that the increase in homologous DNA transformation in UV-DNA experiments we have observed in both *rec*⁺ and repair/recombination mutants is not mediated through a *rec*-independent process such as an increase in the binding or uptake of DNA.

In all of our experiments described above, DNA purified from undamaged cells is used to transform recipients which have been treated with UV radiation. Since under natural conditions the DNA available to transforming cells probably comes from cells, living or dead, that have been exposed to the same damaging environment as the recipients, an important question is whether there is any benefit to competent cells in taking up and exchanging this equally damaged DNA. To address this, transforming DNA was isolated from a competent culture that had been exposed to UV radiation (50 J/m²) or not and this DNA was used as donor in subsequent UV-DNA and DNA-UV experiments with the *rec*⁺ strain. Like our previous results (8,9), and using donor DNA isolated from undamaged competent cells as control (Fig. 1a), we find a similar qualitative difference in the rate of transformation (about 2 fold at a fluence of 25-50 J/m² to the recipients) between the two treatments when competent cells are transformed with DNA isolated from damaged competent cells (Fig. 1b). There is no statistical difference between experiments in which the donor DNA was either damaged or undamaged. The difference between the UV-DNA and DNA-UV regressions is highly significant ($P=.0001$).

These results show that there is still a benefit to transformation even when the transforming DNA is damaged. In experiments using undamaged donor DNA, it might be suggested that by giving undamaged donor DNA to damaged recipients, we are rigging the experiment to get the observed effect. However, Figure 1 shows that the effect remains even when the donor DNA is as equally damaged as the recipient's DNA. This result suggests that transformation is targeted to damaged sites. If this were not the case and donor DNA was recombined in at random, then there should be no net advantage to transformation in the experiments in which donor and recipient DNA was equally damaged. This would be the case since some of the time damaged donor DNA will have been recombined for good recipient DNA and some of the time good donor DNA will have been recombined for bad recipient

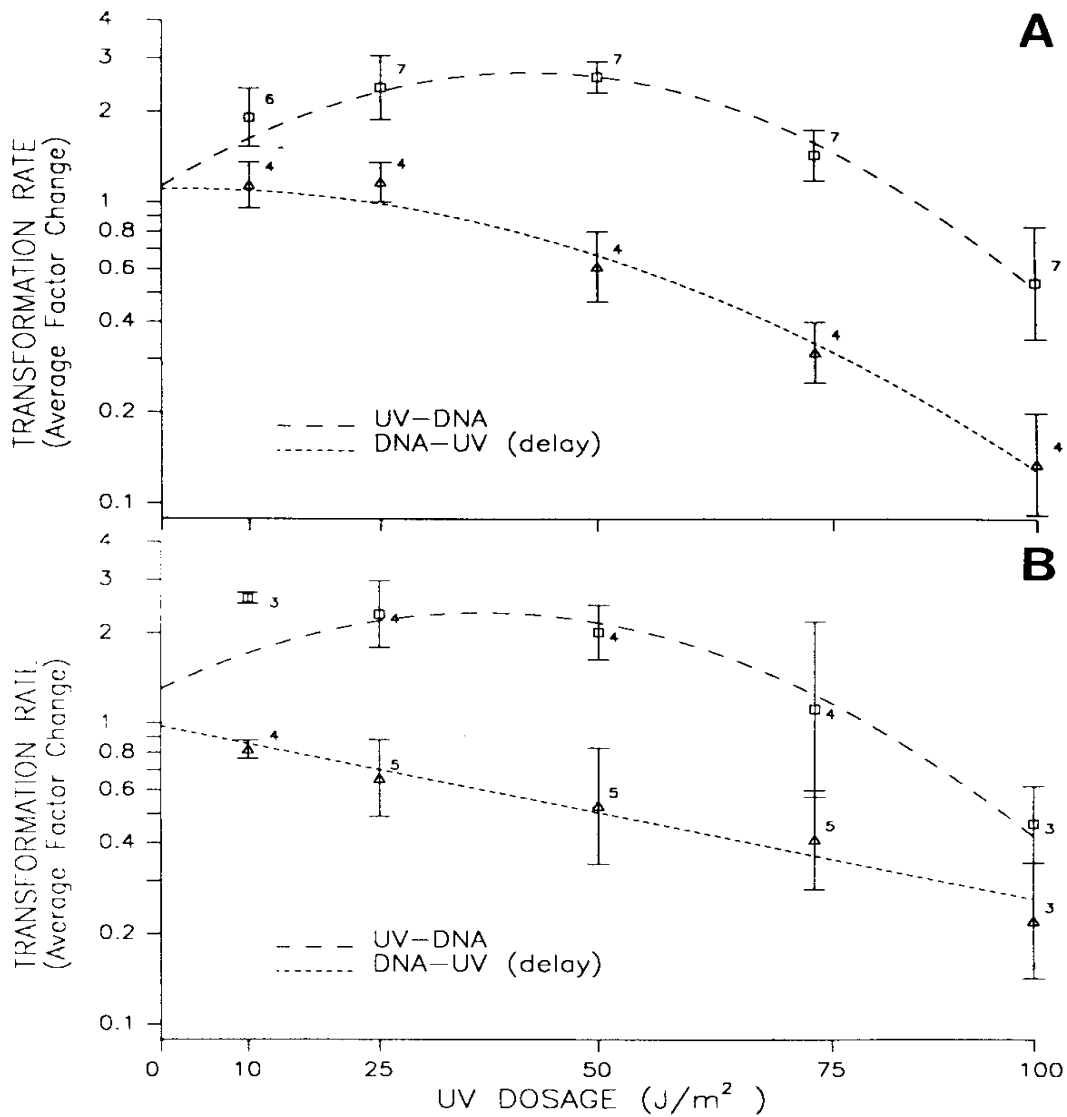


Figure 1. Factor change in frequency of homologous DNA transformation for *B. subtilis recE⁺* strain as a function of UV dosage for UV-DNA and DNA-UV treatments. (A) Transformation using donor DNA purified from undamaged competent cells (B) Transformation using donor DNA purified from damaged (UV irradiated at 50J/m²) competent cells. Plots are averages of the log factor change for each experiment. Error bars give average \pm the standard error, sample size is given near each mean. Regressions are as follows. Figure 1A: DNA-UV regression: $y = 0.04377 + 0.00061x - 0.00010^* x^2$; UV-DNA regression: $y = 0.05321 + 0.01794^{***} x - 0.00021^{****} x^2$. Figure 1B: DNA-UV regression: $y = -0.00573 - 0.01015^{****} x$; UV-DNA regression: $y = 0.11315 + 0.01374^* x - 0.00019^{**} x^2$. * $P < .05$, ** $P < .01$, *** $P < .001$, **** $P < .0001$.

DNA. However, if recombination is targeted to damaged sites in the recipient's genome, damaged donor DNA should serve the purpose just as well as undamaged donor DNA. This is because it is highly improbable that both the incoming and recipient cell's DNA will be damaged at the same specific site. We are now seeking molecular confirmation of this interpretation that recombination is targeted to damaged sites.

REFERENCES

1. Weismann A (1889). "Essays Upon Heredity and Kindred Biological Problems". Clarendon: Oxford.
2. Bernstein H, Hopf FA, Michod RE (1984). Origin of sex. *J Theor Biol* 110:232-351.
3. Bernstein H, Hopf FA, Michod RE (1987). The molecular basis of the evolution of sex. *Adv Genet* 24:323-370.
4. Eigen M, Schuster P (1979). "The Hypercycle, A Principle of Natural Self-Organization". Berlin: Springer-Verlag.
5. Michod RE (1983). Population biology of the first replicators: on the origin of the genotype, phenotype and organism. *Amer Zool* 23:5-14.
6. Stewart GJ, Carlson CA (1986). The biology of natural transformation. *Ann Rev Microbiol* 40:211-235.
7. Dubnau, DA (1982). Genetic transformation in *Bacillus subtilis*. In Dubnau DA (ed): "The Molecular Biology of the Bacilli", Vol. 1. New York: Academic Press, pp 148-175.
8. Michod, RE, Wojciechowski MF, Hoelzer MA (1988). DNA repair and the evolution of transformation in the bacterium *Bacillus subtilis*. *Genetics* 118:31-39.
9. Wojciechowski MF, Hoelzer MA, Michod RE (1989). DNA repair and the evolution of transformation in *Bacillus subtilis*. II. Role of inducible repair. *Genetics* 121:411-422.
10. Love PE, Lyle MJ, Yasbin RE (1985). DNA-damage-inducible (*din*) loci are transcriptionally activated in competent *Bacillus subtilis*. *Proc Natl Acad Sci USA* 82:6201-6205.