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International Institute for
Applied Systems Analysis
Schlossplatz 1
A-2361 Laxenburg, Austria

Tel: +43 2236 807 342
Fax: +43 2236 71313
E-mail: publications@iiasa.ac.at
Web: www.iiasa.ac.at

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Koji Yahara (kyahara@ims.u-tokyo.ac.jp)
Ryota Horie (horie@riken.u-tokyo.ac.jp)
Ichizo Kobayashi (ikobaya@ims.u-tokyo.ac.jp)
Akira Sasaki (sasaki_akira@soken.ac.jp)

Approved by

Ulf Dieckmann
Leader, Evolution and Ecology Program

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Contents

Evolution of DNA double-strand break repair by gene conversion: coevolution between a phage and a restriction-modification system

Koji Yahara[*], Ryota Horie[‡], Ichizo Kobayashi*[,†], and Akira Sasaki[§,#]

[*] Laboratory of Social Genome Sciences, Department of Medical Genome Sciences, Graduate School of Frontier Science & Institute of Medical Science, University of Tokyo, Tokyo 108-8639, Japan

[†] Graduate Program of Biophysics and Biochemistry, Graduate School of Science, University of Tokyo, Tokyo, Japan

[‡] Laboratory for Language Development, RIKEN Brain Science Institute, Saitama 351-0198, Japan

[§] Department of Biology, Faculty of Sciences, Kyushu University, Fukuoka 812-8581, Japan

[#] Evolution and Ecology Program, International Institute for Applied Systems Analysis, Laxenburg, Austria

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Author for correspondence: Ichizo Kobayashi

Address: Laboratory of Social Genome Sciences, Department of Medical Genome Sciences, Graduate School of Frontier Science & Institute of Medical Science, University of Tokyo, Tokyo 108-8639, Japan

Phone: +81-3-5449-5326

Facsimile: +81-3-5449-5422

E-mail: ikobaya@ims.u-tokyo.ac.jp

ABSTRACT

The necessity to repair genome damage has been considered to be an immediate factor responsible for the origin of sex. Indeed, attack by a cellular restriction enzyme of invading DNA from several bacteriophages initiates recombinational repair by gene conversion if there is homologous DNA. In the present work, we modeled the interaction between a bacteriophage and a bacterium carrying a restriction enzyme as antagonistic coevolution. We assume a locus on the bacteriophage genome has either a restriction-sensitive or a -resistant allele, and another locus determines whether it is recombination/repair-proficient or -defective. A restriction break can be repaired by a co-infecting phage genome if one of them is recombination/repair-proficient. We define the fitness of phage (resistant/sensitive and repair-positive/-negative) genotypes and bacterial (restriction-positive/-negative) genotypes by assuming random encounter of the genotypes, with a given probabilities of single and double infections, and the costs of resistance, repair and restriction. Our results show the evolution of the repair allele depends on b_1 / b_0, the ratio of the burst size b_1 under damage to host cell physiology induced by an unrepaired double-strand break to the default burst size b_0 . It was not until this effect was taken into account that the evolutionary advantage of DNA repair became apparent.

Sex can be defined as the homology-based transfer of genetic information between DNAs (MICHOD and LEVIN 1988; SANTOS *et al.* 2003; TURNER and CHAO 1998). More specifically, it can be defined as homologous recombination involving out-crossing and crossing-over. In this sense, sex is widely found from prokaryotes to eukaryotes. Its prokaryotic examples include incorporation of incoming DNAs in natural transformation in several bacteria and homologous recombination of bacteriophage genomes by bacteriophage function.

The necessity to repair damage on the genome using undamaged genetic material as a template has been considered to be an immediate factor responsible for the origin of sex (BERNSTEIN *et al.* 1984; LONG and MICHOD 1995; MICHOD and LONG 1995; MICHOD 1998). Recombination genes may have arisen in the first instance because of their role in repair, and this may have remained their major function until today. Indeed, many experiments have demonstrated that homologous recombination is stimulated by damage to DNA. Transformation frequencies in *Bacillus subtilis* increased with increasing levels of DNA damage when the cultures are given homologous DNA (MICHOD and WOJCIECHOWSKI 1994). A DNA double-strand break is repaired by copying homologous DNA, with and without associated crossing-over, in *Escherichia coli* by lambdoid bacteriophages (KOBAYASHI and TAKAHASHI 1988; TAKAHASHI and KOBAYASHI 1990).

However, the repair hypothesis does not readily explain the origin and maintenance of sex in eukaryotes, which is defined as meiotic crossing-over built in the haploid-diploid cycle (BARTON and CHARLESWORTH 1998; MAYNARD SMITH 1988). Previous studies of the evolution of the haploid-diploid cycle showed that the origin and maintenance of this cycle could be solely explained by faster removal of recurrent deleterious mutations in haploids and greater resistance to genetic damage in diploids (KONDRASHOV and CROW 1991; MAYNARD SMITH and SZATHMARY 1995; CAVALIER-SMITH 2002; SANTOS *et al.* 2003). The necessity of repair was not revealed. Furthermore, it is obvious that double-strand repair does not require

meiosiqs and syngamy of sexual reproduction in eukaryotes at all. Indeed, the most popular hypotheses for the evolution of sex in eukaryotes ascribe the advantage of sex to accelerated adaptation to ever-changing environments, which likely result from antagonistic interactions with other organisms, or to efficient elimination of deleterious mutations. A thorough review of this subject has been carried out by KONDRASHOV (1993).

The molecular mechanisms underlying meiotic recombination may provide some clue as to this issue. Meiotic recombination in yeast is initiated by the formation of a double-strand break in one of the numerous sites along the chromosome (KROGH and SYMINGTON 2004). It is repaired by copying a sister chromatid or a homologous chromosome, which may result in gene conversion. This break repair (gene conversion) is often accompanied by crossing-over of the flanking sequences. This led to the hypothesis that the advantage of meiotic recombination is in the elimination of 'non-self' sequences from the genome (KOBAYASHI 1998; TAKAHASHI *et al.* 1997). Similarly, the advantage of sex is hypothesized to be defense against selfish genetic elements (WELCH and MESELSON 2000). The repair hypothesis is strongly related to these hypotheses.

It can be imagined that the costs of sex in the prokaryotes that lack the haploid-diploid cycle are much smaller than those in the eukaryotes, although the machinery for natural transformation appears to be somewhat costly. Therefore, the repair hypothesis can more adequately explain the evolution of sex in the prokaryotes (often called the origin of sex) than in the eukaryotes, although it is not obvious why DNA double-strand break repair has to be often accompanied by crossing-over of the flanking sequences (KUSANO *et al.* 1994) because crossing-over has still a potential to break apart favorable combinations of genes (SHIELDS 1988). However, there are also observations and arguments that question experimental evidence of the repair hypothesis for prokaryotes. One of the observations is that transformation with a small part of the *H. influenzae* chromosome was as effective in increasing survival as with the whole chromosomal DNA (MONGOLD 1992). This result was not predicted by the repair hypothesis bcause the DNA fragment supplied would be able to

patch less than 1% of the possible sites of damage in *H. influenzae* genome. The above mentioned experiments with *Bacillus subtilis* may not have been sufficiently sensitive to detect such modest differences in bacterial survival (REDFIELD 2001).

In the present work, to examine the validity of the repair hypothesis, we focus on sex in bacteriophages in the form of DNA double-strand break repair by gene conversion. A major role of the homologous recombination machinery carried by DNA bacteriophages is suggested to be repair of DNA double-strand breaks made by restriction-modification systems through the double-strand break repair mechanism (TAKAHASHI *et al.* 1997; KOBAYASHI 1998). Attack by a cellular restriction enzyme on invading DNA of several bacteriophages initiates recombinational repair by gene conversion if there is homologous DNA. Because several restriction-modification systems behave as selfish mobile elements, such as transposons and bacteriophages (NAITO et al. 1995; KOBAYASHI 1998, 2004), there is an aspect of biological interaction in this mode of homologous recombination. We model the interaction between a bacteriophage and a restriction-modification system in a bacterium as antagonistic coevolution and explore conditions for sexual (recombination/repair-proficient) phages to evolve by numerical simulations.

As is already suggested by the repair hypothesis, sex in DNA bacteriophages has a cooperative (altruistic) aspect. A repair enzyme of a sexual (recombination/repair-proficient) phage is able to repair not only sexual but also asexual bacteriophage genome if there is a homologous template chromosome for repair. Namely, the DNA repair enzyme can equally act *in cis* and *in trans*, providing an equal opportunity of repair to asexual (recombination/repair-defective) phages. In this case, it can be imagined that evolution of sexual (recombination/repair-proficient) phages is not easy even if the cost of sex is small. Competition between sexual (recombination/repair-proficient) and asexual (recombination/repair-defective) phages in the phage population will become apparent and the former can be viewed as altruistic while the other can be viewed as selfish.

Our simulation revealed that the sexual (recombination repair) allele is able to evolve

only under specific conditions of induced damage to the host cell physiology due to an unrepaired double-strand break. It was not until this effect was taken into account that the evolutionary advantage of DNA repair became apparent.

MODELS

--- Table 1 about here---

--- Figure 1 about here---

We construct a model of the interaction between bacteriophage genomes and a restriction-modification system of a bacterium, in which the survival of an individual with a certain genotype depends on the genotypic frequencies of the interacting species. This is a gene-for-gene system for a bacteriophage genome and a restriction-modification system.

Our model is illustrated in Figure 1, and all the symbols used are explained in Table 1. A bacterial cell either carries a restriction enzyme that can attack a sensitive bacteriophage genome (a^+) or does not carry it (a^-). Each bacteriophage genome has two loci. The first locus (A) harbors either a restriction-sensitive site (A^-) or a restriction-resistant site (A^+). The second locus (Rec) of the bacteriophage harbors either a sexual (recombination/repair-proficient) allele (Rec^+) or an asexual (recombination/repair-defective) allele (Rec^-).

We assume that a bacterial cell may experience no infection at all, may be infected with one phage particle, or may be infected with two phage particles, with predetermined probabilities (P_0, P_1, P_2). The relative proportion of a particular combination of bacteria genotype and infecting bacteriophage genotype(s) is assumed to be given by the product of their frequencies (x, $1-x$, and y_{ij}'s).

Inevitable attack of the restriction enzyme on the restriction site of an invading

bacteriophage genome can initiate recombinational repair of the restriction break by gene conversion if there is a co-infecting phage genome and if at least one of them is recombination/repair-proficient. The probability of successful repair is denoted by r ($r < 1$) when one of the two co-infecting phages is "Rec$^+$" and the other is "Rec$^-$" (Fig 1A). When the co-infecting phages are both Rec$^+$, the probability of repair increases to $2r$ because the amount of Rec enzyme in the host cell is doubled (Fig 1B). If repair succeeds, the "A$^-$" allele of the restricted phage genome is changed to "A$^+$" by gene conversion. Our model assumes that a template chromosome for recombinational repair is supplied only by a co-infecting phage. This assumption of frequent multiple infection is based on the abundance of bacteriophage particles in natural environments (BERGH *et al.* 1989; WALDOR *et al.* 2005). We assume that repair cannot occur in single infection because there is no template chromosome for repair in the bacterial cell.

Undamaged or repaired phage genomes survive and give rise to progeny. We designate the number of progeny as burst size, which is defined as the number of virus particles released per cell (WEINBAUER 2004). As illustrated in Fig. 1, we assume that the burst size decreases when a double-strand break of one of the co-infecting phages remains unrepaired. This assumption is based on the experimental evidence that a single unrepaired double-strand break on a plasmid molecule or a yeast artificial chromosome induces lethality to a cell (BENNETT *et al.* 1993, 1996). We thus introduce another parameter of burst size under induction of damage to the host cell physiology b_1, which is less than or equal to default burst size b_0. Two examples of b_1 / b_0 =1.0 and b_1 / b_0 =0.5 are illustrated in Figure 1. The influence of this parameter is only apparent when co-infection results in survival of one of the infecting phages and death of the other phage with an unrepaired double-strand break. When single infection occurs or co-infection leads to the survival of both phages, any damage is not induced and, therefore, the distinction between b_1 and b_0 is unnecessary. Note that if b_1 / b_0 =1.0, the total burst size is equal to the default burst size b_0 whether the repair succeeds and leading to

the survival of both restriction-sensitive and -resistant phages or it fails and leaving only resistant phage. In the case of successful repair, the two resulting phage genotypes are assumed to give the same number of progeny because there is an upper limit of intracellular resources available in a host cell and they equally share the resources.

There are four genotypes (A^+ Rec^+, A^+ Rec^-, A^- Rec^+, and A^- Rec^-) in the phage population, and two genotypes (restriction-positive (a^+) and -negative (a^-)) in the bacterial population. Phages are sampled randomly from the phage population, with the multiplicity of infection (MOI) from 0 to 2, and allowed to infect one of the two genotypes of bacteria. When no infection occurs in a bacterial cell (MOI=0), or when the restriction-positive bacteria cell is infected by sensitive phage(s), the bacterial cell multiplies.

After single infection either by a "Rec^+" or "Rec^-" bacteriophage, the phage will kill a restriction-negative bacterial cell and produce progeny. On the other hand, a restriction-positive cell will always prevent the growth of a restriction-sensitive phage, but will always yield to a restriction-resistant phage.

Co-infecting phage pairs can be classified into three cases ("Rec^+ and Rec^+" infection, "Rec^+ and Rec^-" infection, and "Rec^- and Rec^-" infection), each of which is further divided into their allelic states at the restriction locus (A^+ or A^-). For each combination, the phages experience three possible events (restriction, repair, and burst).

We assume that there is a cost of carriage of a restriction-modification system on a^+ bacterium, c_1, which is realized as a reduced growth rate. The relative fecundity of a^+ bacterium to that of a^- bacterium depends on the cost of restriction-modification as $S_1 = e^{-c_1}$. Also assumed are the metabolic cost c_2 for restriction-resistance on A^+ phage, and that c_3 for recombination/repair capacity on Rec^+ phage, both represented by a reduced burst size (the relative fecundity, see Table 1). The relative fecundity of A^+ phage is expressed as $S_2 = e^{-c_2}$

and that of Rec$^+$ phage as $S_3 = e^{-c_3}$. If a phage carries both restriction resistant site and Rec allele in its genome, the relative fecundity is given by $S_2 S_3$.

We compile a mating table that contains all the infection patterns, their probability of occurrence and the number of progeny from each pattern. Part of the mating table is shown in Table 2. Note that all the patterns in Table 2 are those for restriction-positive (a^+) bacteria. Other patterns for restriction-negative (a^-) bacteria are not included because they are trivial, in the sense that all the infecting phages survive and thus genotype of their progeny always remains the same as their parents'. The number of phage progeny from an infected bacterium depends on the relative burst size, which is b_0 when both of the co-infecting phages (or the singly infecting phage) survive(s) and b_1 when one of the co-infecting phages survives in the presence of an unrepaired double-strand break of another phage's chromosome. The expected number of phage progeny is assumed to be given by the product of the relative burst size, the relative fecundity depending on the metabolic costs of restriction-resistance and recombination/repair-proficient alleles, and probabilities of each infection and repair. The number of progeny of the host bacterial genotype in the next generation is represented similarly.

From the mating table, we can write down the following equations. The frequency *x* of bacteria which have restriction-modification genes changes between generations as

$$x' = \frac{S_1\left(P_0 + P_1\varphi_0 + P_2\varphi_0^2\right)x}{(1-x)P_0 + xS_1\left(P_0 + P_1\varphi_0 + P_2\varphi_0^2\right)}, \qquad (1)$$

where $\varphi_0 = y_{00} + y_{01}$ is the frequency of restriction-sensitive phages (with $\varphi_1 = y_{10} + y_{11}$, the frequency of restriction-resistant phages). The phage genotype frequencies in the next generation are expressed as

$$
\begin{aligned}
\bar{w}_P y_{00}' &= b_0(1-x)(P_1+P_2)y_{00}, \\
\bar{w}_P y_{01}' &= b_0 S_3(1-x)(P_1+P_2)y_{01}, \\
\bar{w}_P y_{10}' &= b_0 S_2\left[\left\{(P_1+P_2)+P_2 x\left(\frac{2b_1}{b_0}-1\right)\varphi_0\right\}y_{10}\right. \\
&\qquad \left. +rP_2 x\left\{y_{00}y_{11}-\left(\frac{2b_1}{b_0}-1\right)y_{01}y_{10}\right\}\right], \qquad (2) \\
\bar{w}_P y_{11}' &= b_0 S_2 S_3\left[\left\{(P_1+P_2)+P_2 x\left(\frac{2b_1}{b_0}-1\right)\varphi_0\right\}y_{11}\right. \\
&\qquad \left. +rP_2 x\left\{y_{01}(y_{10}+2y_{11})-\left(\frac{2b_1}{b_0}-1\right)(y_{00}+2y_{01})y_{11}\right\}\right],
\end{aligned}
$$

where $\bar{w}_P$ is the mean fitness of phage, which is given by the sum of right hand sides of the above equations.

--- Table 2 about here---

Strong antagonistically interaction between bacteria and phage genotypes represented by frequency-dependent genotypic fitness easily destabilizes an equilibrium of the coupled genotypic dynamics (1)-(2), which, unless the costs of restriction-modification in bacteria and restriction-resistance in phage are too large, show complex limit cycles of large amplitudes. Even when phage population is monomorphic with respect to its recombination/repair locus, the coupled dynamics of restriction-negative and -positive bacteria, and restriction-sensitive and -resistant phages exhibit limit cycles. With periodic oscillation in genotypic frequencies of bacteria and phage population, obtaining the analytical "invasion criteria", the sign of the long term marginal logarithmic growth rate of Rec^+ carrying phage introduced into the resident Rec^- population, becomes difficult.

We therefore numerically explore conditions that allow the sexual (recombination/repair-proficient) allele to evolve. The procedure is summarized in Figure 2,

which is equivalent to the iteration of the recursion (1)-(2) except for the process of mutation described below. After all the combinations are computed based on the mating table, the progeny of each phage/bacterial genotype is summed up to yield the fitness (i.e., expected number of progeny) for all genotypes in each generation. Selection and mutation then operates, resulting in frequency change for each genotype. Mutation is assumed to occur only at the restriction locus of the bacteriophages, which enables us to eliminate the persistence of repair/recombination allele by mutation-selection balance, because we are interested in the adaptive evolution of the repair/recombination allele. This evolutionary process for one generation is repeated for thousands of generations.

--- Figure 2 about here---

The basic parameter values used in our simulation are listed in Table 1. The simulations are extensively carried out by changing the values of b_1, c_3, r, P_2 (and P_1). We then summarize how the condition for the evolution of sexual allele depends on these parameters.

RESULTS

Our simulation gave completely different results for the spread of sex allele depending on the values of b_1 / b_0, although it always gave sustained cycles of genotypes for our choices of parameters. The dependence of the advantage of sex allele on b_1 / b_0 is summarized in Figure 3. Apparently, evolution of the repair allele becomes possible when b_1 / b_0 is small and its cost is small.

When b_1 / b_0 is large, the evolutionary dynamics show victory of Rec$^-$ phages over

Rec^+ phages (Figure 4A). Rec^+ phages continue to decrease in frequency and become extinct even when the initial frequency is very high (99%). The recombination/repair-proficient allele cannot evolve under this condition. Intuitive reason for the failure of sex allele is clear: the damaged sensitive genomes of Rec^- phage can be repaired by co-infecting Rec^+'s enzymes and templates. This implies that Rec^- phage can enjoy advantage of "free-repairs" equally efficiently as altruistic Rec^+ phage does, yet without paying any cost.

Figure 4B shows the relationship between the frequencies of the repair/recombination allele in phages, the restriction-resistant allele in phages, and the restriction-positive strain in bacteria observed in the simulation. More specifically, the inter-generational increase in the frequency of Rec^+ phages, a fitness measure of recombination/repair modifier allele, is plotted against the frequency of a^+ (restriction-positive) bacteria and that of A^+ (restriction-resistant) phages of each generation. The dynamics shows a cycle in which the frequency of a^+ bacteria and that of A^+ phages periodically fluctuate. Apparently, the number of Rec^+ phages consistently decreases, showing no correlated change with the abundance of restriction-resistant phage or restriction-positive bacterium, indicating that no evolutionary advantage of DNA double-strand break repair has been generated under this condition. This makes a sharp contrast with a striking correlated change between Rec^+, a^+ and A^+, which we found in the case of $b_0/b_1<1$ and will be discussed later.

Detailed dynamical interaction between bacteria restriction-positive genotypes and bacteriophage restriction-resistance genotypes is presented in Figure 4C. Among Rec^- phages, the relative frequency of each allele at the restriction-site locus [A^- (restriction-sensitive) or A^+ (restriction-resistant)] shows sustained oscillation. This is also true of Rec^+ phages (data not shown). In the bacteria, the relative frequency of each allele [a^- (restriction-negative) or a^+ (restriction-positive)] alternates in conjunction with the cycles of phage genotypes. These results represent a continuous coevolutionary force acting both on the phage genome and the restriction-modification system in bacteria. When a prevalent genotype of bacteria is a^+

(restriction-positive), A^+ (restriction-resistant) phages survive and spread. Once A^+ (restriction-resistant) phages become prevalent, however, any restriction enzyme of bacteria is no longer effective while its cost still exists. Then a^- (restriction-negative) bacteria increase their frequency in the bacterial population. Once a^- (restriction-negative) bacteria become prevalent, however, A^- (restriction-sensitive) phages in turn have an advantage because resistance of phage genome is no longer useful and becomes costly. The prevalence of A^- (restriction-sensitive) phages makes a^+ (restriction-positive) bacteria advantageous and the dynamics returns to the former state. Sustained cycles of phage and bacteria genotypes are thus produced. Rec^+ modifier allele in phage however consistently decreases as its ability of repairing cleaved restriction-sensitive site benefits co-infecting Rec^- phages equally as well as themselves when b_1 / b_0 is large (equal to or only slightly less than 1).

From these results we were unable to find a definitive evolutionary advantage of the double-strand break repair. However, the results change dramatically as b_1 decreases relative to b_0, as summarized in Figure 5.

The evolutionary dynamics show victory of Rec^+ phages over Rec^- phages, as shown in Figure 5A. Rec^+ phages continue to increase in frequency, even when the initial frequency is very low (1%). This indicates that the double-strand break repair has an evolutionary advantage, enabling Rec^+ phages and the recombination/repair-proficient allele to evolve.

The evolutionary trajectory reveals Rec^+ allele can remarkably increase in frequency by its building up of positive linkage disequilibrium to restriction-resistant sites, as shown in Figure 5B. The sustained cycles of bacteria and phage genotypes then drive the frequency of Rec^+ alleles to fixation. The increase of Rec^+ allele occurs in the phase of cycle in which both a^+ (restriction-positive) bacteria and A^+ (restriction-resistant) phages are prevalent. This indicates that the double-strand break repair between Rec^+ phages has a definitive evolutionary advantage when a^+ bacteria predominate. For sufficiently small b_1 / b_0, the mutually altruistic

repair in Rec^+/Rec^+ infections produces an advantage for Rec^+ by their larger contribution of progeny to the next generation than that in Rec^-/Rec^- infections, which can overcome the Rec-'s advantage of "free-repairs" in Rec^-/Rec^+ heterologous co-infections. This at the same time generates a positive correlation between Rec^+ allele and restriction-resistant allele. Larger contribution of Rec^+/Rec^+ homologous infection is due to its prevention of induced damage to the host cell physiology by the unrepaired double-strand break in the Rec^+/Rec^+ infections, as parameterized in the model as ($b_1 / b_0 < 1$).

The sustained cycle of each genotype [A^- (restriction-sensitive) or A^+ (restriction-positive)] among Rec^+ phages is similar (Fig 5C) to the case when Rec^+ decreases (Fig 4C). Thus apparently same coevolutionary cycles of bacteria restriction-modification genotypes and phage restriction-resistance genotype have quite different effects on the fate of sexual allele in phage -- they can drive the costly sexual allele to fixation if b_1 / b_0 is sufficiently smaller than 1, but fail to do so if b_1 / b_0 is large.

The conditions for the evolution of the recombination/repair-proficient allele also critically depends on the probability P_2 of co-infection and the probability r of successful repair in the presence of Rec^+ phage. The results of extensive simulations shown in Figure 6 demonstrate that the higher are the values of these two parameters, the more likely is the evolution of repair allele. These results indicate that considerable co-infection and repair are necessary for evolution of the double-strand break repair, even when b_1 / b_0 is small.

DISCUSSION

Our simulation gave completely different results depending on b_1 / b_0, the ratio of the burst size b_1 under induced damage to the host cell physiology to the default burst size b_0. It was only when this effect of the induced damage was taken into account that the evolutionary

advantage of the double-strand break repair became apparent. The validity of the repair hypothesis for the origin of sex is, therefore, confirmed under a limited condition.

Under the condition where b_1 / b_0 is high, the repair allele did not increase at all. Namely, double-strand break repair did not show any evolutionary advantage under this condition. This seems counterintuitive, because progeny of the Rec^+ phage indeed increases by repair of its genome in A^- Rec^+/A^+ Rec^- infection and the cost of repair is assumed to be not very large (Figure 3). In our model, however, the DNA repair enzyme equally acts *in cis* and *in trans*, providing an equal opportunity of repair to Rec^- phages. In A^- Rec^-/A^+ Rec^+ infection (Figure 1A), once-restricted Rec^- phage is repaired by enzyme from Rec^+ phage, resulting in a decrease in Rec^+ progeny. Therefore, the benefit of repair for the Rec^+ phages is completely counterbalanced by that of the Rec^- phages. In addition, even in A^- Rec^+/A^+ Rec^+ infection, where both infecting phages are Rec^+ (Figure 1B), double-strand break repair confers no advantage for Rec^+ because repairing genome does not change the total burst size. For example, in Figure 1B, 100 progeny of Rec^+ result from repair failure, while 50 plus 50 progeny of Rec^+ result from repair success. This number is the same as that for progeny of Rec^- phage in A^- Rec^-/A^+ Rec^- infection without any repair. This is why double-strand break repair confers no selective advantage under the condition $b_1 = b_0$.

In contrast, the repair allele did increase from a very low initial frequency when b_1 / b_0 is smaller than 1. As in the case of b_1 / b_0 close to 1, the benefit of repair of Rec^+ in A^- Rec^+/A^+ Rec^- infection is counterbalanced by that of Rec^- phages. However, the fitness difference between Rec^+ and Rec^- phages is generated when A^- Rec^+/A^+ Rec^+ and A^- Rec^-/A^+ Rec^- infections are compared. In A^+ Rec^+/A^- Rec^+ infection, where both the infecting phages are Rec^+, the total progeny of Rec^+ increases by repair success. For example, 50 progeny of Rec^+ result from repair failure, while 50 plus 50 progeny of Rec^+ result from repair success (see Figure 1B). In contrast, in A^- Rec^-/A^+ Rec^- infection where no repair occurs, the progeny of

surviving Rec^- phage decreases to b_1 (50) because a remaining double-strand break of one of the co-infecting phage genomes induces damage to the host cell physiology. This represents the definitive advantage of repair for Rec^+ phages under this condition.

The disadvantage of A^- Rec^-/A^+ Rec^- infection means Rec^- is recessive deleterious. Therefore, Rec^- phages decreased slowly as they became rare in the population because the probability of Rec^-/Rec^- co-infection became lower. After the initial 10000 generations, Rec^- phages decreased its frequency from 99% to about 17%. After the next 10000 generation, however, Rec^- phages did not become extinct and decreased more and more slowly (less than 0.1%).

In our model, there is no fitness difference between Rec^+ and Rec^- phages when single infection or infection to a^- bacteria occurs. This is also true of co-infection in which both phages are A^- or A^+. We therefore examined our results in the above explanations by focusing on A^+/A^- co-infection.

The evolutionary dynamics of Rec^+ and Rec^- was smooth compared to that of A^+ and A^-. alleles as was in Fig. 4 or Fig. 5. In contrast, if a genetic correlation between modifer (Rec) and selected (A) locus is close to 100% and recombination rate between them is close to 0%, frequencies of modifier alleles (Rec^+ or Rec^-) strongly depend on those of selected alleles (A^+ or A^-). This corresponds to a situation where a modifier (Rec) locus sits very close to a selected (A) locus and recombination between them does not occur. Although the situation is possible if there are some restriction sites in a genome, our model assumed one restriction site (A) for simplicity. Therefore, modifier (Rec) locus did not gain an association with a selected (A) locus and frequencies of modifier alleles (Rec^+ or Rec^-) changed more slowly than those of selected alleles (A^+ or A^-). Selection coefficient of modifier locus is the squared order of that of selected locus (ISHII *et al.* 1989).

The predominance of Rec^- phages under a large b_1/b_0 condition is caused by complementation. Co-infection of a virus supplying a gene product leads to a defective virus

gene that is then represented in the progeny, which instead decreases the progeny of the former functional virus (DENNEHY and TURNER 2004; FROISSART *et al.* 2004; NOVELLA *et al.* 2004). This is apparently disadvantageous for the functional viruses (Rec^+ phages in our model), which can be viewed as altruists, while the defective (Rec^-) phages can be viewed as free-riders or cheaters (MAYNARD SMITH and SZATHMARY 1995; KELLER 1999; FOSTER *et al.* 2004). Meanwhile, the condition of small b_1 / b_0 selectively benefits Rec^+ progeny on Rec^+/Rec^+ infection, as shown in Figure 5B. The repair process under competition between co-infecting phages is considered to act as a mechanism that constrains cheaters (TRAVISANO and VELICER 2004). Our model represents one of the mechanisms for constraining cheaters in microbes (FOSTER *et al.* 2004). Although cheating, cooperation and sociality in microbes have not been the focus of attention until recently, these are now being pointed out as fundamental issues in evolutionary theory and in pathogenicity control (SMITH 2001; FROISSART *et al.* 2004; GRIFFIN *et al.* 2004; TRAVISANO and VELICER 2004).

We assume that repair-defective (Rec^-) phages can produce progeny in the absence of a bacterial recombination system (RecBCD). In lambdoid bacteriophages, packaging of the phage genome into a viable phage particle needs a concatemer form, in which phage DNA units are joined together in a head-to-tail manner (FUJISAWA and MORITA 1997). Formation of the concatemer is blocked by the RecBCD DNase of the host *Escherichia coli*, which degrades non-self DNA but repairs self DNA marked by an ID sequence (HANDA *et al.* 2000; HANDA *et al.* 1997). Lambda and other bacteriophages produce an inhibitor of RecBCD DNase (SMITH 1983). Therefore, our model corresponds to the RecBCD-negative states.

In reality, even a single infecting phage genome might encounter homologous prophage genomes in the host cell that can serve as a template for repair. Prophages are abundant in the sequenced bacterial genomes. For example, a natural isolate of *Escherichia coli* carries 18 prophages and phage remnants, among which 13 are related to bacteriophage lambda (HAYASHI *et al.* 2001). In addition, an infecting bacteriophage may start replication before attack by a certain type of restriction enzyme, which seems to produce a template for

repair of a sister chromosome (HANDA and KOBAYASHI 2005). These effects might provide an additional advantage of double-strand break repair in that a single infecting "A^- Rec^+" bacteriophage is able to revive to some extent after attack by a restriction enzyme, which would selectively benefit Rec^+ phages while eliminating cheaters.

The burst size of a once-restricted and repaired bacteriophage could be lower than that of an undamaged phage on the assumption that an infecting bacteriophage would start replication before attack by certain types of restriction enzyme, as explained above. Accordingly, the restriction and repair process would delay replication of the bacteriophage, which could in turn increase progeny of the undamaged coexisting phage. Our simulation does not explicitly include this effect. However, we already confirmed that the effect could not change the result because Rec^+ and Rec^- phages had equal opportunities to increase their progeny by this effect.

Our model was constructed in the framework of evolutionary game theory: a powerful tool in both social science and evolutionary biology to analyze social problems involving interdependence among several agents (MAYNARD SMITH 1982; NOWAK and SIGMUND 2004). It is now recognized as being applicable to social interactions such as cheating and cooperation in microbes as well (TURNER and CHAO 1999; KERR *et al.* 2002; NOWAK and SIGMUND 2002; WOLF and ARKIN 2003; PFEIFFER and SCHUSTER 2005; TURNER 2005; WOLF *et al.* 2005). It has been claimed that one of the most important challenges lying ahead is to model the interaction of strategies encoded in genomic sequences (NOWAK and SIGMUND 2004). Our model represents one of the first examples of such attempts (see also MOCHIZUKI *et al.* 2006).

Our one-locus model has been simplified from the gene-for-gene model used by SASAKI (2000), which assumed multi-locus and asymmetric gene-for-gene interaction. This simplification enabled us to write down all the interactions between bacteriophages and host bacteria into a simple mating table, even if we also consider a modifier locus (Rec) and co-infection. Multi-locus models yield a much greater number of genotypes and of interactions between them, which makes analysis and interpretation difficult. Despite the simplification,

our model similarly showed protected genetic polymorphism in the genotype of the host (phage genome in our model) and the parasite (restriction-modification system in bacteria in our model) and produced a sustained cycle of genotype frequencies. This is a robust tendency in many gene-for-gene models, which has been considered to give an advantage to recombination and sexual reproduction, although the cycle itself has not been experimentally proven (see, for example, Korona *et al* (1993)). To the best of our knowledge, this work represents the first study examining whether these characteristics of the dynamics enable sex (recombination repair) in bacteriophages to evolve. Because our one-locus models cannot distinguish between gene conversion not associated with flanking crossing-over and gene conversion associated with flanking crossing-over, whether the dynamics yield a short-term advantage for crossing-over remains an unexplored question.

The repair process of our model is assumed to begin only after a double-strand break by a restriction enzyme, which is similar to the "damage-induced sex" proposed by Michod and colleagues (LONG and MICHOD 1995; MICHOD and LONG 1995; MICHOD 1998). However, they assumed different molecular mechanisms, in that gene damage was repaired by cell or proto-cell fusion with damaged or undamaged partners. These differences lead to different results, especially in a situation in which sexual cells mate with asexual (cheater in our model) cells (MICHOD and LONG 1995).

It is conceivable that the repair mechanism used in our model represents one form of bacteriophage adaptation to attack by restriction enzymes (KOBAYASHI 1998), that is, an example of anti-restriction strategies (TOCK and DRYDEN 2005). An advantage of the mechanism, however, only becomes obvious when a remaining double-strand break of one of the co-infecting phage genomes induces damage to the host cell physiology, resulting in a decrease in the burst size, although some additional advantages might exist as well.

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FIGURE LEGENDS

Figure 1. Model. (A) Co-infection of a restriction-positive bacterial cell with an "A^+ (restriction-resistant) Rec^+ (repair-positive)" phage and an "A^- (restriction-sensitive) Rec^- (repair-negative)" phage. Co-infection occurs with predetermined probability P_2 multiplied by frequencies of bacteria x and that of phage y_{ij} (see Table 1 for the symbols). The "A^- Rec^-" phage genome is cut at the A^- site by the restriction enzyme. The Rec^+ enzyme can repair the double-strand break by copying the A^+ allele with a probability of r. The "A^-" locus is converted to "A^+" by gene conversion. If repair is successful, the undamaged "A^+ Rec^+" phage and the repaired "A^+ Rec^-" phage give the same number of progeny. If repair fails with a probability of $1-r$, only the "A^+ Rec^+" phage gives the progeny. The default burst size common to a single infection and a multiple infection is b_0. When a double-strand break of one of the co-infecting phage genomes remains unrepaired, the burst size would be reduced to b_1 by induction of damage to the host cell physiology. The above explanation and those for the other infection patterns are summarized in the mating table of Table 2. **(B) Co-infection of a restriction-positive bacterial cell with an "A^+ Rec^+" phage and an "A^- Rec^+" phage.** When co-infecting phages are both Rec^+, the probability of repair increases to $2r$ because the amount of Rec enzyme in the host cell is doubled.

Figure 2. Simulation. There are four genotypes (A^+/A^-, $Rec^+/^-$) in the phage population, while the bacterial population has two genotypes (restriction-positive and -negative) as listed in Tables 2. From the phage population, phages are sampled with MOI from 0 through 2 and allowed to infect one of the two types of bacteria. For each combination, the phages experience three events (restriction, repair, burst). After all combinations are computed, progeny of each phage/bacterial genotype is summed to yield fitness (i.e., expected number of progeny). Selection and mutation then operates, resulting in a frequency change for each genotype. This

evolutionary process for one generation continues for thousands of generations.

Figure 3. Conditions for evolution of repair/recombination allele. Phase diagram of the metabolic cost of repair/recombination enzyme c_3 and the ratio of burst size under induced damage to the host cell physiology b_1 to default burst size b_0 . Other parameter values are listed in Table 1. A black dot (•) represents victory of Rec^+ over Rec^- phage. A gray square (■) represents victory of Rec^- over Rec^+ phage. A white triangle (△) represents an unsettled case after 30 000 generations: neither Rec^+ or Rec^- became extinct.

Figure 4. Results for large b_1/b_0. Results for $b_1 = b_0 = 1.0$ and $c_3 = 0.001$. Other parameter values are listed in Table 1. **(A) Evolutionary dynamics indicating victory of Rec^- phages over Rec^+ phages.** Rec^- phages increase in frequency. The initial frequency of Rec^- phages is 1% (0.5% $A^- Rec^-$ and 0.5% $A^+ Rec^-$). **(B) Evolutionary trajectory indicating continuous decrease of Rec^+ phages.** X-axis indicates the frequency of a^+ (restriction-positive) bacteria of each generation, and y-axis indicates that of A^+ (restriction-positive) phages. Z-axis indicates the inter-generational increase in the frequency of Rec^+ phages. These values are plotted until 20000 generation by black dots and the trajectories of about one cycle from 5000, 7500, and 19000 generation are illustrated by a black, green, and blue line respectively. The frequency of Rec^+ phages continues to decrease from the initial frequency (99% as in Fig. 4A) while the frequency of a^+ bacteria and that of A^+ phages periodically oscillate. The trajectory is counterclockwise. **(C) Periodic oscillation of restriction-sensitive and -resistant genotypes of Rec^- phages in the interaction with bacterial dynamics.** The vertical axis indicates the frequency of $A^- Rec^-$ and that of $A^+ Rec^-$ phages along with the frequency of a^- and that of a^+ bacteria in the total population. The frequency of each genotype of Rec^- phages [A^- (restriction-sensitive) or A^+ (restriction-positive)] oscillates in the interaction with bacterial

genotypic dynamics [a^- (restriction-negative) or a^+ (restriction-positive)], where Rec^- phages increase in frequency.

Figure 5. Results for small b_1/b_0. Results obtained for b_1/b_0=0.5 and c_3=0.001. Other parameter values are listed in Table 1. The composition is the same as for Figure 4. **(A) Evolutionary dynamics indicating victory of Rec^+ phages over Rec^- phages.** Rec^+ phages increase in frequency. The initial frequency of Rec^+ is 1% (0.5% A^- Rec^+ and 0.5% A^+ Rec^+). **(B) Evolutionary trajectory in which Rec^+ allele can remarkably increase.** X-axis indicates the frequency of a^+ (restriction-positive) bacteria of each generation, and y-axis indicates that of A^+ (restriction-positive) phages. Z-axis indicates the inter-generational increase in the frequency of Rec^+ phages. The initial frequency of Rec^+ phages is 1% and a typical trajectory is illustrated from 5546 to 5606 generation by a black line, while other parts are the same as Fig. 4B. Rec^+ phages remarkably increase in frequency only when increase of a^+ (restriction-positive) bacteria and ensuing increase of A^+ (restriction-positive) phages occur. **(C) Periodic oscillation of restriction-sensitive and -resistant genotypes of Rec^+ phages in the interaction with bacterial dynamics.** Sustained cycles of genotypic dynamics appear as shown in Figure 4C, except that the frequency of Rec^+ phages gradually increases due to the altruistic repair in Rec^+/Rec^+ infections.

Figure 6. Parameter dependence for evolution of recombination allele when b_1/b_0 is low. Phase diagram with respect to co-infection probability and repair probability when P_0 is fixed, b_1/b_0=0.5 and c_3=0.001: **(A) wide view**; and **(B) close-up**. Other parameter values are listed in Table 1. The symbols used are as for Figure 3.