Evolution of Bacterial Transformation: Is Sex With Dead Cells Ever Better Than No Sex at All?

Rosemary J. Redfield[1]

Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts 02138

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ABSTRACT

Computer simulations of bacterial transformation are used to show that, under a wide range of biologically reasonable assumptions, transforming populations undergoing deleterious mutation and selection have a higher mean fitness at equilibrium than asexual populations. The source of transforming DNA, the amount of DNA taken up by each transforming cell, and the relationship between number of mutations and cell viability (the fitness function) are important factors. When the DNA source is living cells, transformation resembles meiotic sex. When the DNA source is cells killed by selection against mutations, transformation increases the average number of mutations per genome but can nevertheless increase the mean fitness of the population at equilibrium. In a model of regulated transformation, in which the most fit cells of a transforming population do not transform, transforming populations are always fitter at equilibrium than asexual populations. These results show that transformation can reduce mutation load.

GENETIC transformation occurs naturally in a number of groups of bacteria, including *Micrococcus*, *Haemophilus* and *Bacillus*. Under appropriate environmental conditions cells become competent to take up homologous DNA from the environment and recombine it into their genomes, replacing the endogenous copies of the sequences taken up [see STEWART and CARLSON (1986) for a recent review]. Natural transformation systems are usually considered to be specially evolved attributes of cells, unlike the artificially inducible transformation of microorganisms such as *Escherichia coli* and yeast (LOW and PORTER 1978). Transformation may have been selected for a function other than recombination. For example, BERNSTEIN *et al.* (1985) and MICHOD, WOJCIECHOWSKI and HOELZER (1988) have suggested that the evolutionary function of transformation may be to provide template strands for DNA repair. Alternatively, transformation may simply allow bacteria to acquire nucleotides for use in DNA synthesis (STEWART and CARLSON 1986). It is also possible that transformational DNA uptake is an unselected side effect of mechanisms whose primary cellular functions have not yet been identified.

With the possible exception of transformation, there is little evidence that bacteria have been selected for the ability to exchange genes. The two other "parasexual" processes of bacteria (plasmid-mediated conjugation and phage-mediated transduction) appear to transfer bacterial genes only accidentally, by mechanisms specialized for transfer of the plasmid or phage genome into new host cells (LEVIN and LENSKI 1983). Similarly, physical recombination of homologous DNA strands in bacteria is carried out by enzymes whose primary function appears to be DNA repair rather than recombination (WALKER 1985).

Although natural transformation and meiotic sex are usually assumed to confer similar evolutionary advantages, the evolutionary function of meiotic sex is itself not well understood [see MAYNARD SMITH (1978) and BELL (1982) for reviews]. The possible long-term advantages of genetic variability to the species may be insufficient to explain the origin and short-term persistence of sexual reproduction in individuals. Short-term advantages of sex appear to depend on extreme environmental fluctuations (CHARLESWORTH 1976), small populations (MULLER 1964; HAIGH 1978), or fitness-decreasing interactions between mutations (ESHEL and FELDMAN 1970). Selection against the accumulation of deleterious mutations is the most ubiquitous factor giving an advantage to sexual reproduction (CROW 1970; KONDRASHOV 1982).

Unlike eukaryotic sex, where both partners contribute equally to the genome of the progeny, transformation is not reciprocal; there is a donor of genetic material (DNA source) and a recipient (transforming cell). The natural sources of transforming DNA are unknown. Although there is some evidence that *Bacillus subtilis* cells may actively secrete DNA (SINHA and IYER 1971), for other bacteria the most plausible donors are dead conspecific cells. Because the ge-

[1] Present address: Department of Molecular Biology and Genetics, Johns Hopkins University School of Medicine, 725 N. Wolfe St., Baltimore MD 21205

nomes of dead (donor) cells will on average carry more deleterious mutations than those of the surviving (recipient) cells, the selective costs of transformation may be higher than those of meiotic sex.

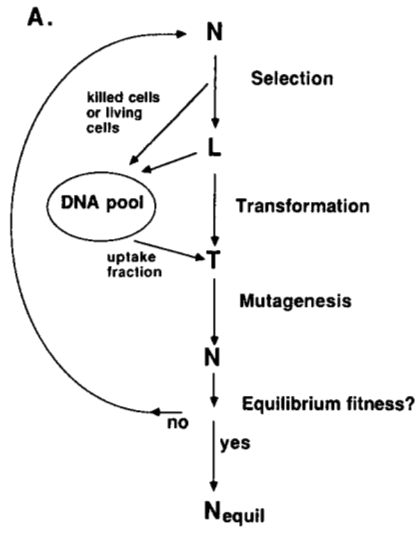
Mechanisms of natural transformation have been widely studied in the laboratory, but until recently little attention has been paid to the selective forces responsible for their evolution. We know very little about transformation under natural conditions (although see GRAHAM and ISTOCK 1981), and theoretical studies of possible selective factors are lacking. In order to identify the true evolutionary causes of transformation we must first understand the various ways it can affect fitness. To this end I have developed a computer simulation model of bacterial transformation and used it to investigate the effect of transformation on mutation load.

THE MODEL

The basic model (Figure 1*A*) starts with a large mutation-free population of bacteria, and follows the accumulation of deleterious mutations until an equilibrium is reached where selection, transformation and mutagenesis are in balance. In each generation new mutations are first added to the cells' genomes according to a preset mutation rate. All mutations are unique and equally deleterious. The probability of a cell surviving the subsequent selection step depends on the fitness function and the number of mutations in its genome. Cells that survive selection reproduce, restoring the population to its original size. In simulations where living cells are the source of transforming DNA, each cell then releases a copy of its genome into the environmental DNA pool, where the DNA is broken into fragments of a specified size. The size of these transforming fragments determines the amount of genetic exchange. If the DNA source is dead cells, the genomes of the cells killed by selection are similarly released and fragmented. Each living cell then takes up a single randomly selected DNA fragment which replaces the homologous segment of its own genome. Linkage between loci is ignored. Because the transforming fragment can contain more or fewer mutations than the genomic segment it replaces, transformation can change a cell's mutation burden.

The selection and mutagenesis steps are based on the model of HAIGH (1978). The relative frequencies of cells with different numbers of mutations after selection, transformation and mutagenesis are given by the mutation-distributions **L**, **T** and **N** respectively; the *i*th term of each distribution gives the frequency of cells with i mutations (i ranges from 0 to *imax*).

Selection: The fitness distribution **V** gives the fraction of each mutation class (each N_i) that survives selection, as a function of i and of s, the selective cost



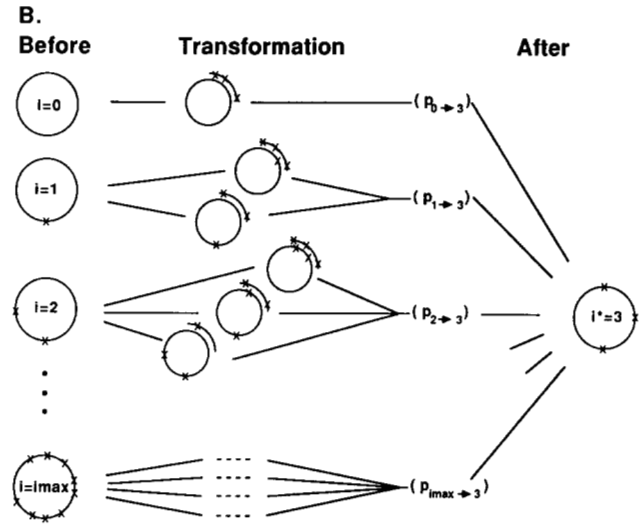


FIGURE 1.—A, Outline of the model used in this study. **N**, **L** and **T** are the mutation distributions of the population after mutagenesis, selection and transformation respectively. B, Paths of transformation that produce cells having three mutations; see text.

of a single mutation. Two types of fitness functions were investigated:

$$V_i = (1 - s)^{i^a} \quad \text{and} \quad V_i = 1 - (is)^{\alpha}.$$

These are described in more detail in the RESULTS section. The normalized distributions of mutations among the survivors of selection and the cells killed by selection are given by **L** and **K**, respectively, where

$$L_i = \frac{N_i V_i}{\sum N_i V_i} \quad \text{and} \quad K_i = \frac{N_i(1 - V_i)}{\sum N_i(1 - V_i)}.$$

Normalization of **L** (the population that will undergo transformation) is equivalent to a reproduction step. **K** is used when the killed cell population is to be the source of transforming DNA.

Transformation: The amount of DNA in the environment is assumed to be not limiting for transformation. The size of the DNA fragments taken up by transforming cells (specified as a fraction of the genome, r) sets the amount of recombination. In the model it affects the distributions of mutations in both the DNA pool fragments and the genome segments that these fragments replace. The distribution of mutations in the genome is assumed to be binomial, so the probability that a fragment or segment of length r derived from a genome with i mutations will contain j mutations is $(j^i)rj(1 - r)^{i-j}$. These probabilities (for any given r) are stored as the i,j^{th} terms of a matrix **B**.

Simulation of transformation requires two steps: (1) calculation of the distribution of mutations in the DNA pool fragments (distribution **D**), and (2) calculation of the mutation distribution in the cells after transformation with this DNA (distribution **T**). **D** is calculated (after selection) as the product of **B** with **L** or **K**. Each D_j is the sum of the fraction of donor cells with i mutations (L_i or K_i) multiplied by the probability that such cells will yield fragments with j mutations ($B_{i,j}$):

$$D_j = \sum_{i=0}^{\text{imax}} L_i B_{i,j} \quad \text{or} \quad D_j = \sum_{i=0}^{\text{imax}} K_i B_{i,j}.$$

Calculation of **T** is more complex. Two components of transformation can change a cell's mutation number: replacement of mutations in a genome segment by wild-type sequences from the homologous DNA fragment (curing), and replacement of wild-type genome sequences by mutations from the fragment. A cell that begins transformation with i mutations will finish with i^* mutations if the fragment it takes up cures it of j mutations (probability $B_{i,j}$) and also adds $i^* - (i - j)$ mutations (probability $D_{i^*-(i-j)}$), so the net probability of transformation from i to i^* is

$$p_{i-i^*} = \sum_{j=\max(0,i-i^*)}^{i} B_{ij} D_{i^*-(i-j)}.$$

(When $i - i^* < 0$ the summation must start as $j = 0$ so that indices remain non-negative.) T_{i^*}, the total fraction of the population transforming to i^* mutations, is then the sum of these probabilities for cells with $i = 0, 1, 2 \ldots imax$ initial mutations, each multiplied by L_i, the fraction of the population that it represents:

$$T_{i^*} = \sum_{i=0}^{imax} L_i p_{i-i^*}.$$

Figure 1B shows schematically the paths by which cells with $i^* = 3$ mutations can arise. No normalization step is needed after transformation because the population size is unchanged.

Mutagenesis: Assuming that mutations arise at random, the probability **M** that a cell will acquire k new mutations at each generation is a Poisson distribution whose mean is the genomic mutation rate U. Back mutation is ignored (*cf.* HAIGH 1978). The mutation distribution of the population after mutagenesis, **N**, is accordingly calculated from the transformed-cell distribution **T** as:

$$N_i = \sum_{k=0}^{imax} T_{i-k} M_k.$$

Mean fitness: The mean fitness of the cells in the population (the fraction of cells that will survive the next round of selection) is calculated after each generation as

$$W = \sum_{i=0}^{imax} N_i V_i$$

and a simulation is considered to have reached its equilibrium distribution when fitnesses in successive generations differ by less than 10^{-8} (commonly less than 200 generations were required).

The amount of computation per generation depends strongly on *imax*, the maximum number of mutations considered per genome and per DNA fragment. To minimize running time and to prevent truncation errors, *imax* was usually set at 20, and the selective cost per mutation (s) and genomic mutation rate (U) chosen so that the last terms of the mutation distributions were smaller than 10^{-8} (when necessary, *imax* was increased to 30 or 35). Most simulations were done at $U = 1$ and s between 0.05 and 0.5.

Changing the assumptions: Uptake of small DNA fragments from multiple donors was simulated by binomially redistributing the mutations within the DNA pool before transformation. The effect of DNA availability was tested by simulations in which only half of the cells in each mutation class transform. Persistence of DNA in the environment (half-life one generation) was simulated by adding each generation's DNA pool distribution to that of the previous generation, and normalizing.

RESULTS

Testing the model: When $r = 0.5$ and the DNA donors are living cells (DNA pool calculated from the **L** mutation distribution) the genetic consequences of transformation should be the same as those of meiotic sex. This allows the model described above to be tested by comparing the results using living-cell DNA with those of previously-reported models of sexual reproduction.

MAYNARD SMITH (1968) has shown analytically that when each additional mutation decreases individual fitness by a constant factor (multiplicative selection;

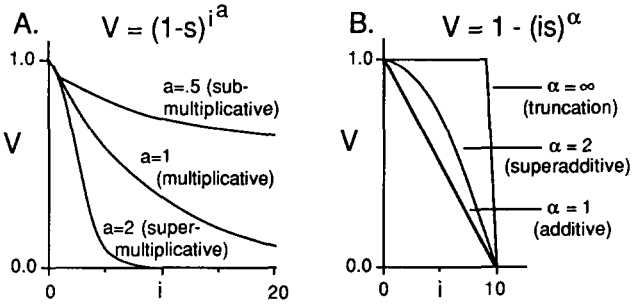


FIGURE 2.—Fitness functions. i = mutations per genome, V = individual fitness; a and α are epistasis coefficients; s is a selective coefficient. s = 0.1. A, Multiplicative fitness functions; B, additive fitness functions.

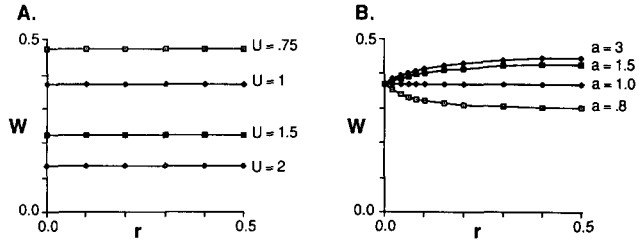


FIGURE 3.—Equilibrium fitnesses (W) of populations as a function of DNA uptake (r) for different fitness functions. DNA source is living cells. A, Multiplicative selection: each line shows identical data for s = 0.1 and s = 0.3. B, Multiplicative fitness functions (U = 1.0, s = 0.2).

$V_i = (1 - s)^i$, Figure 2A, middle line), populations with sexual and asexual reproduction have the same mean fitness at equilibrium. Figure 3A shows that the equilibrium fitness of transforming populations under multiplicative selection is similarly independent of the amount of recombination (the amount of DNA taken up). In further agreement with sexual models, the equilibrium fitness depends on mutation rate U but not on s (HALDANE 1937) and equals e^{-U} (the probability of receiving no new mutations) as predicted by CROW (1970). The transformation step does not change the mutation distribution at each generation (**T** = **L**), and so the number of generations to equilibrium and the mutation distribution at equilibrium are unchanged.

Results of this model also agree with those of a model of eukaryotic sex where sex increases population fitness. KONDRASHOV (1982, 1984) has investigated the relationship between sexual recombination and the cost of deleterious mutations, using fitness functions based on $V_i = 1 - (i/k)^\alpha$ (where $k = 1/s$). Examples of these additive functions are shown in Figure 2B, for k = 10 (s = 0.1). Using computer simulations Kondrashov has found that sexual populations are fitter than asexual populations when α equals 1, 2 and ∞, with larger α giving a larger advantage. Transformation has been simulated using the values of α, k and U tested by KONDRASHOV (1982, Table 2: U = 0.5, 2 and 8; k = 5 and 20, with r = 0.5. The transformation and sexual recombination models give identical values of asexual and sexual equilibrium fitness, mean mutation number at equilibrium, and advantage of sexual reproduction, confirming that this model of transformation with DNA from living cells is, as predicted, comparable in its consequences to eukaryotic sexual recombination.

Dependence on interactions between mutations: As indicated above, the effect of sex on population fitness depends on the interactions we assume between mutations at different loci. ESHEL and FELDMAN (1970) have shown analytically that when beneficial mutations at two loci are segregating, recombination is neutral for multiplicative fitness, advantageous when the fitness of the double mutant is less than the product of the fitnesses of the single mutants, and disadvantageous when the fitness of the double mutant is greater than that product. This suggests that the effects of recombination on population fitness might be best understood using fitness functions based on multiplicative interactions (Figure 2A) rather than the additive interactions studied by KONDRASHOV (1982) and shown in Figure 2B. Accordingly the original multiplicative fitness function was modified to give

$$V_i = (1 - s)^{i^a}$$

where a is an interaction or epistasis factor. When $a > 1.0$ additional mutations are increasingly deleterious (super-multiplicative selection), and when $a < 1.0$ additional mutations decrease fitness by smaller factors (sub-multiplicative). Figure 2A shows these functions with a = 2.0, 1.0 and 0.5.

Figure 3B shows the consequences of transformation using this function with different values of a(U = 1.0 and s = 0.2). The effect (advantage or cost) of transformation increases with increasing DNA uptake to a maximum effect at r = 0.5, then decreases symmetrically to give the asexual fitness (= zero effect) when r = 1.0. When a = 0.8, transforming populations have lower equilibrium fitness than asexual populations, and when a = 1.5 and 3.0 transforming populations are more fit than asexual ones. More generally, the advantage of transformation increases as the fitness function becomes more steeply supermultiplicative (increasing a), and transformation becomes more costly as each additional mutation becomes less harmful (a decreasing below 1.0). With every tested value of a greater than 1.0 transformation increases population fitness, and with every a less than 1.0 transformation decreases fitness (both a = 0.99 and a = 1.01 have been tested).

Varying the mutation rate U has two major effects. One is to shift curves on the y-axis to the new asexual fitness as seen in Figure 3A, and change the mean number of mutations per cell. In addition, the relative

effect (cost of advantage) of transformation increases with increasing mutation rate for both $a = 0.5$ and $a = 2.0$ (not shown). Varying s, the selective cost per mutation, does not alter the asexual fitness, but decreasing s increases the effect of transformation and also increases the time to equilibrium and the number of mutations per cell at equilibrium. Changes in s and U do not alter the qualitative effects of the fitness function: recombination is always neutral when $a = 1$, disadvantageous when $a < 1$, and advantageous when $a > 1$.

Transformation with DNA from dead cells: Some if not all of the DNA available to transforming cells probably comes from cells that have died due to natural selection. Because cells killed by the most recent selection contain on average more mutations than do the survivors of selection, transformation with their DNA (distribution **K**) should therefore increase the average number of mutations in the recipients. Simulations show that although the equilibrium fitness of transforming populations using dead-cell DNA is always lower than that of corresponding populations using living-cell DNA, transformation can still increase fitness above the asexual level (Figure 4). Under conditions where transformation with living cell DNA does not increase equilibrium fitness ($a \leq 1.0$ or $\alpha = 0.8$), transformation with DNA from dead cells always reduces fitness below that of the corresponding asexual population. However, with most super-multiplicative fitness functions, populations transforming with DNA from dead cells are fitter than asexual populations when r is small, but less fit when larger fractions of the genome are taken up and replaced.

Figure 4A shows this effect for multiplicative fitness functions with different values of a. As a increases so do both the maximum advantage of transformation and the advantageous range of fragment sizes. Increasing a above 3.0 has little additional effect. Figure 4B gives results of simulations using the additive-based fitness functions shown in Figure 2B. The maximum advantage is seen with the extreme threshold function ($\alpha = \infty$), and $r = 0.06$–0.08 of a genome, and transformation continues to increase fitness above the asexual value for $r \leq 0.4$. The advantage of transformation is much higher than that seen with multiplicative functions in Figure 4A. The model gives similar advantages with the (super-multiplicative) quadratic fitness functions described by Crow (1970).

The relationship between the fitness function and the mean fitness of the population at equilibrium is not as simple as when DNA comes from living cells, where transformation increases equilibrium fitness whenever the fitness function is super-multiplicative. With DNA from dead cells both the existence and the magnitude of the increase in mean fitness are

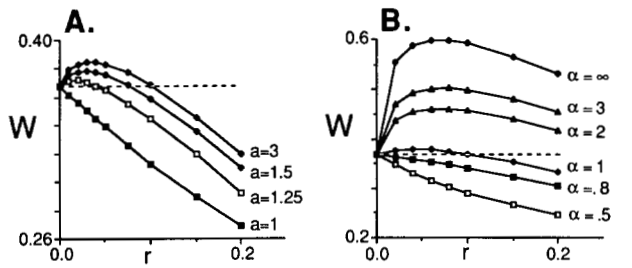


FIGURE 4.—Equilibrium fitnesses (W) of populations transforming with DNA from dead cells; $U = 1.0$, $s = 0.1$. *Dashed lines* show fitness of asexual population. A, Results with multiplicative fitness function; B, results with additive fitness functions.

further constrained by s and U. Decreasing s increases the advantage (both the range of uptake and the advantage at the optimum). Decreasing U increases the equilibrium fitness of the population but shrinks the advantageous range of r. With moderate mutation rates ($U = 0.1$) an advantage is seen only with uptake of very small fragments, and only with steeply convex fitness functions or small s. If a population has a very low mutation rate ($U = 0.01$) transformation with DNA from dead cells decreases its equilibrium fitness.

Threshold-regulated transformation: In the above models, the entire population undergoes transformation every generation. However, the probability that a cell will increase its fitness by transformation depends on its initial state—the more mutations a cell already has, the greater the chance that transformation will cure it of some of them. Most important, a mutation-free cell can never decrease its burden of mutations by transformation, but does risk increasing it by acquiring mutations in the DNA it takes up. In real bacteria, activities which are advantageous under some physiological conditions and disadvantageous under others are usually found to be regulated by the state of the cell (Miller and Reznikoff 1978; Gottesman 1984). In the context of this model, the advantage of transformation might be increased if competence for transformation were regulated by cell fitness or by the number of mutations in the genome. This was simulated by introducing into the model a threshold for transformations, so that in each generation only the fraction of the population with at least a threshold number of mutations participates in the DNA uptake step, although all cells continue to contribute to the DNA pool.

Examples of the results are shown in Figure 5. Figure 5A shows the equilibrium fitnesses of populations with different thresholds. Selection is by a sub-multiplicative fitness function ($a = 0.8$), and transformation is with DNA from living cells. Under these conditions unregulated transformation (threshold = 0) decreases mean fitness at equilibrium for all values of r, but transformation with any positive threshold is advantageous. The largest advantage is

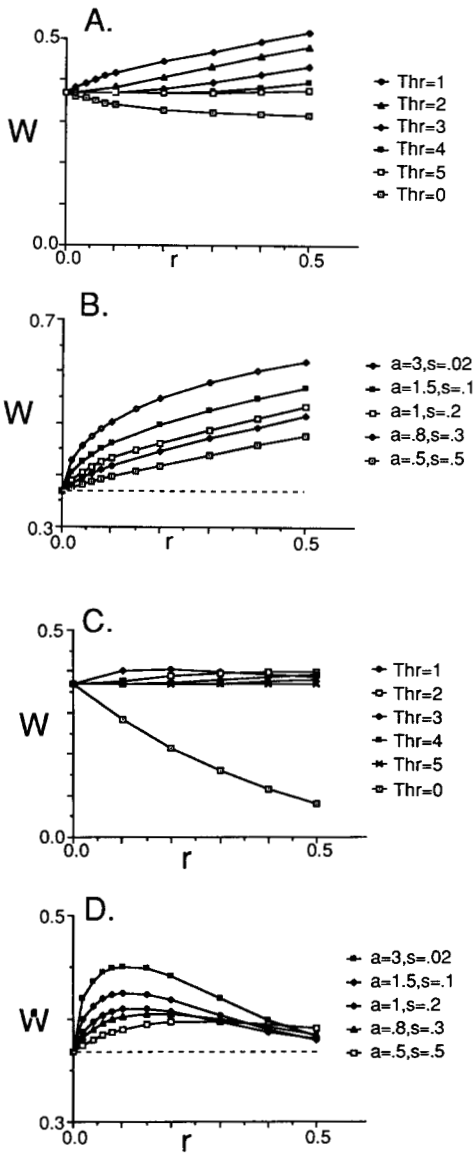


FIGURE 5.—Equilibrium fitnesses (W) of populations with threshold-regulated transformation. $U = 1.0$. A and B, DNA from living cells; A, effect of varying threshold ($a = 0.8$, $s = 0.3$); B, effect of varying a (threshold = 1, $s = 0.2$). C and D, DNA from dead cells: C, effect of varying threshold ($a = 0.8$, $s = 0.3$); D, effect of varying a (threshold = 1, $s = 0.2$).

seen with threshold = 1, and in each case the advantage increases with increasing r. Figure 5B shows the results for selection with different fitness functions and a competence threshold of 1. The advantage is smaller with the sub-multiplicative fitness functions ($a = 0.5$ or 0.8), but mean fitness at equilibrium is always higher than that of an asexual population (shown by the dashed line). Similar results are seen with higher thresholds.

With killed cells as the DNA donors, regulated transformation again always increases mean fitness at equilibrium. Although the advantage of transformation may be small, especially where the threshold is high or large fragments of the genome are replaced, mean fitness never falls below the asexual value. Figure 5C shows equilibrium fitnesses for a range of thresholds from 0 to 5, with $a = 0.8$. Figure 5D shows the results for a range of values of a, with a threshold of 1. The maximum DNA uptake shown is $r = 0.5$, but equilibrium fitness remains above the asexual fitness for all values of r smaller than an entire genome.

I have also simulated threshold-regulated transformation with additive and quadratic fitness functions (KONDRASHOV 1982; CROW 1970). Transformation with a competence threshold of 1 or higher always increases equilibrium fitness above the asexual value of e^{-U}, regardless of the values of the epistasis factors (not shown). With living-cell DNA the fitness increases with increasing recombination; with dead-cell DNA fitness initially rises with increasing recombination and then falls as r becomes large; in no case does fitness ever fall below the asexual value.

Changing the assumptions of the model: The model is robust; changing any of a number of assumptions does not alter the qualitative results. To simulate cells taking up many small sub-fragments of DNA from different donors, the DNA pool was randomized before each transformation; this usually changes the mean fitness at equilibrium by no more than 1%. The effect of limiting DNA was simulated by allowing only half of the population to transform each generation; this has approximately the same effect as halving r. Persistence of DNA in the environment (half-life one generation) slows the approach to equilibrium but does not change the final mean fitness. Simulations in which the DNA pool contains equal parts of DNA from the living and dead populations give equilibrium fitnesses that are midway between those of the pure populations.

DISCUSSION

How can sex with dead cells be better than no sex at all? The higher equilibrium fitness of populations transforming with dead-cell DNA appears counterintuitive. The reason may be understood when the effects of transformation with dead-cell DNA are considered as a combination of the recombination advantage seen with living-cell DNA transformation (but experienced regardless of the DNA source), and the additional mutation load the cells suffer due to incorporation of mutation-laden DNA from dead cells (shown schematically in Figure 6). When r is small the recombination advantage dominates, because this advantage increases rapidly with small uptake and more slowly with larger r (Figure 3B). When larger fragments are taken up the mutation cost (which increases linearly with r) dominates.

The simulations using DNA from living cells serve two functions. Their agreement with models of

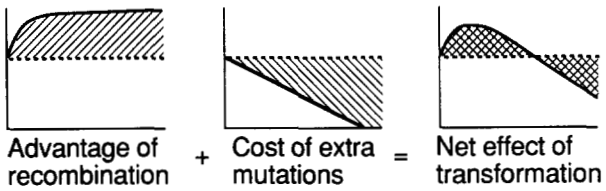


FIGURE 6.—Effects of transformation with DNA from dead cells.

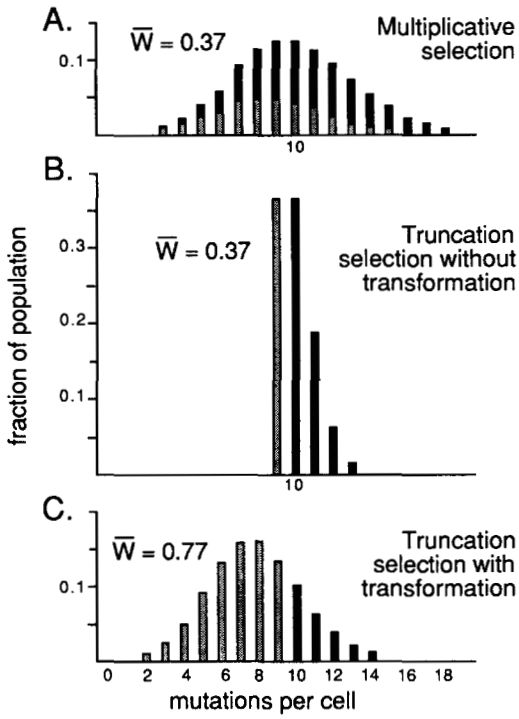


FIGURE 7.—Equilibrium mutation distributions of populations (U = 1.0). The grey area of each bar is the fraction of that class that will survive selection; the sum of these is the mean fitness of the population. A; Multiplicative selection; s = 0.1. B and C, Truncation selection. A and C, Transformation with DNA from living cells; r = 0.5. B, No transformation.

meiotic sex confirms that the model accurately simulates recombination. They also allow us to see how the advantage of recombination depends on the fitness function. The dependence appears to arise because transformation counteracts the skewing that nonmultiplicative selection imposes on the mutation distribution. This can be most clearly understood by reference to Figure 7, which shows what happens when a population initially at equilibrium with multiplicative selection becomes subject to the truncation fitness function ($\alpha = \infty$) shown in Figure 2B. At the initial equilibrium (Figure 7A) fitness is 0.37 and mutations are randomly distributed around the mean in both transforming and asexual populations (because they arise at random and are treated independently by selection and recombination). The equilibrium population fitness is the fraction of the population that would survive the next round of selection, indicated by the gray area of each bar. Now introduce truncation selection with truncation at 10. In the absence of transformation, population fitness will be high for the first few generations, because few cells have ten or more mutations. However, at equilibrium (Figure 7B) all of the population will have nine or more mutations, and the population fitness will have returned to e^{-U} = 0.37, because only those cells which receive no new mutations are viable. Recombination prevents this extremely skewed distribution from arising, because after each round of selection the mutation distribution is partially or completely randomized. Figure 7C shows the mutation distribution and population fitness at equilibrium for a transforming population under the same selection as in Figure 7B. With less extreme super-multiplicative fitness functions the magnitude of the effect is smaller but the causes and consequences are similar. Sub-multiplicative selection exerts its effects in the same way, but because the mutation distribution becomes skewed in the other direction, when transformation randomizes the distribution the equilibrium population fitness is decreased.

Advantage of threshold-regulated transformation: The most striking consequence of threshold regulation is that, even when the mutation cost to the cells that transform is very high, the fitness of the whole population never falls below the asexual level of e^{-U}. This can be understood by considering the extreme case where r = 1 and the DNA source is dead cells (as if every transforming cell replaces its entire genome with that of a cell killed by selection). With truncation selection (e.g., the $\alpha = \infty$ function shown in Figure 2B) transformation is lethal because every fragment in the DNA pool caries a lethal number of mutations. With threshold-regulated transformation the asexual mutation-free class then benefits by the elimination of the mutant classes which compete with it, and the equilibrium fitness of the population is e^{-U}, the fraction of the mutation-free class that remains mutation free.

Threshold regulation is also able to overcome the recombination cost imposed by sub-multiplicative fitness functions. As the function becomes increasingly sub-multiplicative (as a or α decreases) the recombination cost increases, but so does the difference in individual fitness between cells with zero and one mutation ($V_0 - V_1$). This increasing fitness difference increases the advantage of protecting the mutation-free cells from transformation, and thus compensates for the increased recombination cost. The advantage of protecting mutation-free cells from transformation can be much larger than predicted by the size of this class in the absence of threshold regulation, because the class is augmented each generation by those

previously-mutant cells cured of their mutations by transformation.

Applicability of the model to natural transformation: Real mutations do not obey fitness functions, and little is known about the average effects of mutations in any organism. MUKAI (1969) found that the fitnesses of multiply mutant Drosophila could be approximated by a super-multiplicative quadratic function, $V = 1 - 0.01i - 0.005i^2$, whereas mutations in the *Escherichia coli lacY* and *lacZ* genes interact submultiplicatively (DYKHUIZEN, DEAN and HARTL 1987). In principle, whenever regulatory mechanisms depend on the interactions between genes or gene products, additional mutations might be expected to have increasingly deleterious effects.

Laboratory measurements of the frequency of loss-of-function mutations in bacterial genes extrapolate to genomic mutation rates of 0.001–0.002 per generation (DRAKE 1974), considerably lower than the $U = 1.0$ used in most of the above simulations. If natural rates are this low, the mean fitness of asexual populations will be almost independent of deleterious mutations (mean fitness at equilibrium = 0.999), and recombination would have very little effect. There are several reasons to expect mutation rates under natural conditions to be much higher than 0.001. (1) The measured mutation rates probably underestimate actual rates under laboratory conditions, but by no more than a factor of four (DRAKE 1974). (2) Laboratory rates are more likely to more seriously underestimate the evolutionarily significant rates because bacteria are very poorly shielded from mutagenic substances and radiation present in the natural environment but not in laboratory cultures. (3) Theoretical considerations discussed by COX (1976) and MAYNARD SMITH (1978) suggest that selection against deleterious mutations would not have been able to decrease mutation rates to this low level. Although unregulated transformation with dead-cell DNA is disadvantageous when U is less than about 0.1, both regulated transformation and transformation with living-cell DNA increase equilibrium fitness of populations with all non-zero mutation rates.

Might transformation be induced by accumulation of deleterious mutations? Most natural transformation is inducible, in that rapid deterioration in growth conditions can lead to large transient increases in the efficiency of DNA uptake, often by 10^3 or more (KAHN and SMITH 1984), but the environmentally significant inducers have not been identified. Although a mutant gene is not physically distinguishable from a wild-type gene, cells do have various metabolic surveillance systems which detect deterioration of the intracellular environment and induce appropriate changes in gene expression; some of these detect very broad classes of disturbances (GOTTESMAN 1984). These regulatory networks will also respond when the metabolic changes are caused by mutations, for example in amino acid biosynthetic pathways or DNA repair enzymes. Thus in a constant environment (assumed in this model) the main effect of gene regulation will be survival of cells with deleterious mutations.

The amount of DNA taken up by competent cells under laboratory conditions is fairly small; a maximum of 10% of the genome for Micrococcus, and 5% for Haemophilus (SMITH, DANNER and DEICH 1981). Because only one strand of each DNA fragment participates in recombination, this uptake will result in net replacement of only a few percent of the genome, well within the most advantageous range under most conditions tested.

The assumption that all mutations are distinct (by descent and by kind) inflates the advantage of recombination, but because a real bacterial genome contains several thousand genes and simulations were usually limited to a maximum of 20 mutations this is unlikely to be a significant source of error. Similarly, linkage effects will not be important if transforming bacteria normally take up many small fragments of DNA. Because the advantages seen result from recombination within single populations, they are not inconsistent with the clonal population structures observed for two naturally transforming bacteria (MUSSER *et al.* 1985; CAUGANT *et al.* 1987).

A possibly significant factor not addressed by this model is the physiological costs of competence and DNA uptake. The development of competence requires changes in cell physiology which usually substantially decrease cell viability. Cell walls become thin and weak, and lysis is frequent. In addition, uptake of homologous or heterologous DNA can cause induction of endogenous defective prophages in the recipient genome, leading to cell death (SETLOW *et al.*, 1973).

Small asexual populations incur disproportionately high mutation loads because genetic drift can lead to the irreversible loss of the cells with the lowest number of mutations (Muller's ratchet) (MULLER 1964; HAIGH 1978). Small sexual populations are able to regenerate mutation-free cells by recombination and thus avoid this cost. The transformation model assumes a large population so that stochastic effects such as genetic drift can be ignored. While this assumption allows many components of the transformation process to be efficiently simulated, Muller's ratchet does not advance. The advantages of transformation will probably appear substantially greater when small asexual and transforming populations are compared in a stochastic model.

Evolution of genes for transformation: The primitive condition in the evolution of transformation was

probably unregulated uptake of small amounts of DNA spontaneously released by dead cells, and the model shows that this transformation can decrease mutation load at equilibrium. These differences should correspond to differences in competitive ability of populations, because fitness relationships do not reverse during the approach to equilibrium. More complex models will be needed to show (1) whether selection against deleterious mutations can allow a newly-arisen gene for transformation to invade an asexual population and (2) whether, once DNA uptake and recombination became established, a gene causing DNA release by living cells will be selected.

Before the true evolutionary function of transformation can be understood the theoretical constraints on other possible selective advantages of transformation (such as DNA repair) will have to be determined, and much more information obtained about the biology of natural transformation. The molecular signals leading to induction of competence must be identified, and the natural sources of transforming DNA characterized. Analysis of the structure and regulation of the genes responsible for transformation should yield valuable information about the selective forces that shaped them.

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