NICHE EXPANSION IN BACTERIA: CAN INFECTIOUS GENE EXCHANGE AFFECT THE RATE OF EVOLUTION?

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ABSTRACT

Recombination occurs by infectious gene transfer in bacteria, at rates much lower than recombination by sexual reproduction in other organisms. Thus, recombination may accelerate evolution in bacteria only under restricted conditions, such as occur when mutations at several loci are required for the evolution of an expanded ecological niche. Mathematical ("chemostat") models of several such cases—evolution of independence from three limiting essential or "interactive-essential" resources; evolution of the ability to use three new substitutable resources; and evolution of resistance to three growth inhibitors—were analyzed by computer simulation. All combinations of three mutation rates (U) and four values for the "infectious gene transfer rate parameter" (χ) were considered. Recombination accelerated evolution most when U was low and χ was high, but was unlikely to have large effects when χ was low enough to be realistic for natural populations of *Escherichia coli*. Recombination had the largest effects when resources were substitutable, and in that case could have substantially reduced the chance of random loss of the favored "triple mutant" while it was still rare. The simulations also revealed some interesting features of selection for an expanded niche. Evolution of independence from essential resources occurred more rapidly when the resources were weakly complementary than when they did not interact. Selection for the ability to use all substitutable resources was weak after all intermediate types that used only one or two of the resources had arisen.

GENETIC recombination cannot change the frequencies of individual genes, but it can change the frequencies of the multilocus genotypes made up of those genes. FISHER (1930) and MULLER (1932) were the first to suggest that this effect might cause populations of sexually reproducing organisms to evolve faster than populations of asexual organisms. They reasoned that sex could assemble a multilocus genotype faster from existing mutants in many lineages than mutation could add favorable genes to the genotype of a single lineage. Since then, the details of this line of argument have been developed. A series of special cases have been studied, and some important generalizations have emerged. MAYNARD SMITH (1968) and ESHEL and FELDMAN (1970) found that recombination would not speed the fixation of a favored two-locus genotype if the population was initially in linkage equilibrium. KARLIN (1973) found that recombination could speed the fixation of a favored

two-locus genotype if the two favored alleles were negatively associated initially. CROW and KIMURA (1965, 1969) and MAYNARD SMITH had argued, with less mathematical rigor and generality, that recombination could speed evolution in finite populations because newly arising mutants at different loci would generally be negatively associated. FELSENSTEIN (1974) iced the cake by arguing that effects of genetic drift [specifically the "Hill-Robertson" effect (HILL and ROBERTSON 1966)] would tend to generate negative associations between favored alleles at different loci, suggesting that recombination could accelerate evolution quite generally.

These studies all focused on recombination as it occurs in sexually reproducing organisms, and it has commonly been assumed that recombination in bacteria is too weak to have substantial quantitative effects on rates of evolution (LEVIN and LENSKI 1983; BODMER 1970). Yet, no one has explicitly modeled recombination as it occurs in bacteria with the goal of studying its effects, or lack thereof, on evolutionary rates. Could even the weak recombination of bacteria accelerate their evolution, at least in some circumstances?

The rates of recombination caused by infectious gene transfer are certainly much lower than most rates of sexual recombination. When a two-factor cross, $a^+b^- \times a^-b^+$, is performed by mating an *Escherichia coli* F^+ strain with an F^- strain, the number of a^+b^+ cells obtained is roughly the same as the number arising in either strain by mutation alone (LEDERBERG and TATUM 1946a, b; LEWIN 1977). Thus, the rate of recombination caused by transmission of the F plasmid is very low, on the order of the mutation rate at best. Moreover, studies of the population structure of *E. coli* in mammalian guts argue against high rates of recombination under natural conditions. SELANDER and LEVIN (1980) found strongly nonrandom associations of alleles at 20 loci examined electrophoretically in *E. coli* isolated from natural sources. Despite a high mean genetic diversity ($H = 0.47$), seven groups of two to four clones were identical for all 20 loci, a virtually impossible result if electromorphs were randomly distributed. CAUGANT, LEVIN and SELANDER (1981) studied *E. coli* populations in a single human over 11 months and found nonrandom associations between alleles at several pairs of loci. Both groups of authors argued that strong epistasis between many arbitrarily chosen loci is unlikely, so these results strongly suggest that rates of recombination in nature are also low, on the order of the mutation rate or less.

Perhaps specific ecological situations cause selection to act in ways that would allow these tiny amounts of recombination to have effects. If selection is driving a bacterial strain to expand its ecological niche—for instance, to become independent of several resources that limit its growth, to use a new set of resources or to become resistant to several inhibitors—a number of genetic changes may be required, and mutation alone may be very slow in making them. Thus, even a small amount of recombination might be enough to speed the process. In this paper I consider four models of niche expansion in bacteria, incorporating infectious gene transfer at low rates, in an attempt to answer this question.

METHODS

The model considers three loci, with two alleles at each locus. Since I am modeling bacterial populations, all organisms are assumed to be haploid. All cells with a particular three-locus genotype, *ijk*, are members of "strain ijk." A "$\hat{}$" above a letter indicates that the strain being considered carries the alternate allele to the one carried by strain ijk at that locus. For example, strain $\hat{\imath}jk$ carries the alternate allele at the first locus to that carried by strain ijk. A dot in place of a letter indicates that strains with either allele at that locus are being considered together. Thus, $i\cdot\cdot$ indicates that all those strains with the "*i*" allele at the first locus are being considered, regardless of the alleles carried at the other loci. When I refer to particular strains, the two alleles possible at each locus are symbolized by "*1*" *or* "*2*," so that strain 121 carries the "*1*" allele at the first locus, "*2*" at the second, and "*1*" at the third.

The basic model is

$$\frac{dB_{ijk}}{dt} = \phi_{ijk}B_{ijk} + M_{ijk} + R_{ijk} - \rho B_{ijk}, \tag{1}$$

where B_{ijk} is the population density of strain ijk, t is time (hrs), ϕ_{ijk} is its growth rate, M_{ijk} is the rate of change in B_{ijk} due to mutation, R_{ijk} is the rate of change in B_{ijk} due to recombination, and ρ is the rate at which cells are lost from the habitat. This is an extension of the "equable" habitat (chemostat) model used by STEWART and LEVIN (1973). Resources and/or inhibitors are continuously supplied from a reservoir to populations of bacteria assumed to be growing in well-mixed liquid medium. Cells and unused resources flow out of the culture vessel at the same rate, ρ, that resources flow in.

MUTATION

The net rate of change in B_{ijk} due to mutation is

$$M_{ijk} = U(B_{\hat{\imath}jk} + B_{i\hat{\jmath}k} + B_{ij\hat{k}} - 3B_{ijk}), \tag{2}$$

where U is the mutation rate per locus (hr^{-1}). Note that I assume that mutation occurs only at one locus at a time, that rates of "forward" and "back" mutation are the same and that the mutation rate is the same at all loci. Since this approach is deterministic, I also assume that once a mutant occurs it will not be lost due to random forces (*i.e.*, no favored mutant can be washed out).

RECOMBINATION

The net rate of change in B_{ijk} due to recombination is

$$R_{ijk} = \chi[B_{ijk}B_{i\cdot\cdot} + B_{ijk}B_{\cdot j\cdot} + B_{ijk}B_{\cdot\cdot k} - B_{ijk}(B_{\hat{\imath}\cdot\cdot} + B_{\cdot\hat{\jmath}\cdot} + B_{\cdot\cdot\hat{k}})], \tag{3}$$

where χ is the "gene transfer rate parameter" defined by LEVIN (1981).

Equation (3) may be hard to understand without some explanation. Cells of

three strains may be converted to strain ijk by gene transfer: strains $\hat{i}jk$, $i\hat{j}k$ and $ij\hat{k}$. All strains carrying the i allele can donate that allele to strain $\hat{i}jk$, so the input into strain ijk from $\hat{i}jk$ is $\chi B_{ijk}B_{\hat{i}}\ldots$ Similar reasoning explains the $B_{i\hat{j}k}B_{\cdot\hat{j}\cdot}$ and $B_{ij\hat{k}}B_{\cdot\cdot\hat{k}}$ terms. Cells of strain ijk itself may be "lost" by transfer of alleles $\hat{i}$, $\hat{j}$ or $\hat{k}$ into ijk. All strains carrying the $\hat{i}$ allele can donate that allele to strain ijk, giving a loss term of $B_{ijk}B_{\hat{i}}\ldots$ The other loss terms can be explained in the same way, considering the other two loci.

Note that I assume that recombination is a one-way transfer of a gene, that only one gene at a time is transferred and that the same recombination rate parameter applies to all pairs of loci. These assumptions are roughly equivalent to assuming that the cells are carrying a conjugative plasmid and that the three loci are not tightly linked. This model would probably describe gene transfer by transduction and transformation as well (LEVIN 1981).

GROWTH

I consider four different ecological cases, which differ in the resources and/or inhibitors present in the environment and in the way in which the effects of different resources on the growth rate are combined.

1. "Strictly essential" resources: This case represents growth when three essential, noninteracting resources, such as amino acids, vitamins or mineral nutrients, the use of which does not depend on the use of any other resource, occur in equally "limiting" concentrations. An increase in the concentration of any one or two resources does not increase growth rate, since at least one resource remains more limiting than the other one or two.

In this case I assume that the growth of a strain is dependent on the concentrations of those resources it cannot synthesize for itself. "Wild type," strain 222, cannot synthesize any of the three resources. The single mutants (the three strains carrying a single "*1*" allele) can synthesize single, different resources and the double mutants (the three strains carrying two "*1*" alleles) can synthesize different pairs of resources. Since the growth of both the single and double mutants is still limited by at least one resource (to the same degree as the wild type), the wild type, single mutants and double mutants all have the same growth rate. The triple mutant, strain 111, can synthesize all three resources, so its growth rate is independent of them and is greater than the growth rate of the other strains.

If we assume that evolution begins with the wild type at its ecological equilibrium, we can ignore all the terms other than M_{ijk} and R_{ijk} in (1), since the single and double mutants grow at the same rate as wild type and, thus, will increase only because of mutation and recombination. In this case, then, I do not need to specify a form for ϕ_{ijk}. Simply assuming a strong selective advantage for the triple mutant will be sufficient for my purposes.

Before the triple mutant arises, this case can be described by these modifi-

cations of (1):

$$\frac{dB_{222}}{dt} = 3U(B_S - B_{222}) + 3\chi[B_S(2B_S + B_D) - 2B_{222}B_D] \quad (4)$$

$$\frac{dB_S}{dt} = U(B_{222} + 2B_D - 3B_S) + \chi[2B_D(2B_{222} + B_D) - B_S(4B_S + B_D)] \quad (5)$$

$$\frac{dB_D}{dt} = U(2B_S - 3B_D) + \chi[2B_S^2 - B_D(2B_{222} + B_S + 4B_D)] \quad (6)$$

$$\frac{dB_{111}}{dt} = 3UB_D + 3\chi[B_D(B_S + 2B_D)], \quad (7)$$

where $B_S = B_{221} = B_{212} = B_{122}$ and $B_D = B_{112} = B_{121} = B_{211}$. The derivation of these equations is given in the APPENDIX. I shall call this case "essential resources selection."

2. "Interactive-essential" resources: This case is analogous to growth when three essential, but slightly complementary, resources, such as those amino acids or vitamins that facilitate each other's use, occur in low, "limiting" concentrations. TILMAN (1982) has called such resources "interactive-essential" resources, and the model presented here preserves his meaning of that description.

It is assumed that the growth of each strain is dependent on the concentration of one carbon source (assumed to be glucose, for convenience in notation here and below) and the concentrations of the interactive-essential resources it cannot synthesize for itself:

$$\phi_{ijk} = V_{max}\left(\frac{A_1}{A_1 + K_{ijk1}}\right)\left(\frac{A_2}{A_2 + K_{ijk2}}\right)\left(\frac{A_3}{A_3 + K_{ijk3}}\right)\left(\frac{S_G}{S_G + K_G}\right), \quad (8)$$

where A_1, A_2 and A_3 are the concentrations of amino acids, S_G is the concentration of glucose, V_{max} is the maximum growth rate (which is the same for all strains), K_{ijkm} is the concentration of resource m at which growth rate is half-maximal (when all other resources are in excess) and K_G is the concentration of glucose at which growth rate is half-maximal. The factors in parentheses, taken individually, are the "Monod" equation for growth divided by V_{max} (MONOD 1949). I assume that $K_G = 4$ μg/ml and $V_{max} = 0.7$ for all strains, as has been experimentally determined for *E. coli* growing in a chemostat (LEVIN, STEWART and CHAO 1977).

If a strain cannot synthesize amino acid m for itself, its K_{ijkm} is 4 μg/ml (an arbitrary assumption to maintain consistency with glucose use). If it can synthesize it, and so requires only a tiny amount for growth, its K_{ijkm} is 10^{-5} μg/ml. The wild type is auxotrophic for all three amino acids; the three single mutant strains are each prototrophic for single, different amino acids; the double mutants are prototrophic for different pairs of amino acids; and the triple mutant is prototrophic for all three.

As a consequence of the differences in strains' amino acid requirements and the fact that the terms in (8) are multiplied, relative fitnesses are multiplicative.

In particular, it is important to realize that the single and double mutants are favored by selection over the wild type, unlike their counterparts in essential resources selection. Fitnesses of the wild type and intermediates relative to one another are density-dependent, since amino acid concentrations decrease with increasing densities of strains that take up the amino acids (see below). In what follows, I shall refer to this as the "amino acids selection" case. My choice of amino acids as the example of interactive-essential resources is largely arbitrary and is not based on any empirical evidence. Many amino acids may not be interactive-essential resources, and certainly many other resources may be.

3. "Substitutable" resources: In this case it is assumed that three carbon sources, such as sugars or simpler organic compounds, are present in addition to glucose. In principle, growth on any one or combination of them is possible for a strain that can use all four. The growth rate is

$$\phi_{ijk} = V_{max}\left(\frac{Q_{ijk1}S_1 + Q_{ijk2}S_2 + Q_{ijk3}S_3 + S_G}{Q_{ijk1}S_1 + Q_{ijk2}S_2 + Q_{ijk3}S_3 + S_G + K_G}\right), \tag{9}$$

where S_1, S_2 and S_3 are concentrations of sugars other than glucose. Q_{ijk1}, Q_{ijk2} and Q_{ijk3} are parameters determining which, if any, of the additional sugars can be used by strain ijk. For example, if the strain can use sugar 1, then $Q_{ijk1} = 1$; if it cannot, $Q_{ijk1} = 0$. The wild type cannot use any of the additional sugars; each single mutant can use a different, single sugar in addition to glucose; each double mutant can use a different pair of sugars; and the triple mutant can use all three additional sugars. My approach here follows SMOUSE (1980).

This case corresponds to TILMAN's (1982) "perfectly substitutable" resources for those strains that can use more than one sugar. Since K_G governs the use of all sugars, I have assumed that all additional sugars are used exactly like glucose, if they are used at all. Relative fitnesses of multiple mutants are weighted sums of the fitnesses of single mutants, since sugar concentrations are added. Relative fitnesses are density dependent, since sugar concentrations decrease with increasing densities of the strains that can use the sugars (see below). This will be called the "sugars selection" case. Again, the choice of sugars as the example of substitutable resources is largely arbitrary.

4. Inhibitors: In this case, three inhibitors, such as antibiotics, and one carbon source (glucose) occur in the culture vessel, and the growth rate is

$$\phi_{ijk} = V_{max}\,(e^{-\Delta_{ijk1}I_1})(e^{-\Delta_{ijk2}I_2})\,(e^{-\Delta_{ijk3}I_3})\left(\frac{S_G}{S_G + K_G}\right), \tag{10}$$

where I_1, I_2 and I_3 are the concentrations of the antibiotics in the culture vessel and Δ_{ijkm} is a parameter describing the sensitivity of the strain to antibiotic m. If a strain is sensitive to antibiotic m, its Δ_{ijkm} is 0.1, and if it is resistant, its Δ_{ijkm} is 0. $K_G = 4$ μg/ml for all strains. The wild type is sensitive to all three antibiotics; the single mutants are each resistant to single, different antibiotics; the double mutants are resistant to different pairs of antibiotics; and, of course, the triple mutant is resistant to all three antibiotics. Since many

inhibitors (including most antibiotics) reduce growth rate without actually killing, and their effects range from none to completely stopping growth, an exponential function seems the most appropriate model for this case. Values for Δ_{ijkm} were chosen to be in rough accord with what is known about antibiotic effects on growth rate (for example, see HANSEN and HUBBEL 1980; LUNDBACK and NORDSTROM 1974), and antibiotic concentrations have been chosen so that the wild type can still maintain itself when it occurs alone.

Relative fitnesses are multiplicative and density independent, since terms in (10) are multiplied and no strain removes antibiotics from the medium (see below). This will be referred to as the "antibiotics selection" case, and of course my choice of antibiotics as the example of an inhibitor is arbitrary.

RESOURCE AND INHIBITOR CONCENTRATIONS

The rate of change in the concentration of interactive essential resource m (*i.e.*, amino acid m in amino acids selection) in the culture vessel is

$$\frac{dAm}{dt} = \rho(C_{Am} - A_m) - E \sum_{ijk} \phi_{ijk} B_{ijk}, \tag{11}$$

where C_{Am} is the concentration of the amino acid in the inflow and E is the "conversion efficiency" (the amount of any given resource needed for a single cell division, in micrograms, assumed to be 5×10^{-7}, an empirically determined value for glucose use by *E. coli* [LEVIN, STEWART and CHAO 1977]). The same conversion efficiency is assumed for all resources and strains merely for consistency. Note that amino acids are used in proportion to the net growth rate, as is appropriate for interactive essential resources.

The rate of change in the concentration of substitutable resource m (*i.e.*, one of the additional sugars in sugars selection) in the culture vessel is

$$\frac{dS_m}{dt} = \rho(C_{Sm} - S_m) - E \sum_{ijk} V_{max} B_{ijk} \left(\frac{Q_{ijkm} S_m}{Q_{ijkm} S_m + K_G} \right), \tag{12}$$

where C_{Sm} is the concentration of sugar m in the inflow. Note that sugars are used in proportion to their contribution to the growth rate of the strain, as is appropriate for substitutable resources.

By analogy with (12), the rate of change in the concentration of glucose in the culture vessel is

$$\frac{dS_G}{dt} = \rho(C_G - S_G) - E \sum_{ijk} V_{max} B_{ijk} \left(\frac{S_G}{S_G + K_G} \right). \tag{13}$$

I assumed that no strain could detoxify the medium, so the concentration of an inhibitor (*i.e.*, an antibiotic in antibiotics selection) in the culture vessel was always equal to its concentration in the inflow.

SIMULATION

For the essential resources selection case a single step Euler method was used to solve (4), (5), (6) and (7) numerically, with a step size of 0.1 hr. For the

other three cases the same method was used to solve (1), (11), (12) and (13) numerically, with a step size of 0.01 hr. The single step Euler method involves rewriting the differential equations to be simulated as difference equations and then solving them for a series of values of t. So long as t is incremented by a sufficiently small amount in each iteration, the solutions match those expected for the differential equations quite closely (BOYCE and DIPRIMA 1977). The programs used in the simulations were written in FORTRAN V and were run on a CDC Cyber computer. Copies of the programs can be supplied to interested readers.

Runs were made with U ranging from 10^{-10} to 10^{-6}, and with χ ranging from 0 to 10^{-10}. Runs began with strain 222 at its ecological equilibrium and with resources at their ecological equilibria for strain 222 growing alone. Strains other than wild type were initially present at their mutation-selection equilibrium levels, assuming 10% selection against the "*1*" allele at each locus, with fitnesses multiplicative. This assumption was made to mimic the effects of "periodic selection" (ATWOOD, SCHNEIDER and RYAN 1951), which holds down the frequency of rare genes not under positive selection—assuming 10% selection is not critical to the model. If the equilibrium density of a strain was less than 0.01 cells/ml, that strain was initially absent. This restriction is equivalent to assuming that the culture vessel has a volume of 100 ml, so that densities of less than 0.01 cells/ml are meaningless. The simulations, then, approximate an abrupt change in environment that causes evolution into an expanded niche.

Changes in a strain's population density due to mutation and recombination were constrained like the initial population densities. If the absolute value of the change in B_{ijk} due to mutation and recombination for a single iteration was less than 0.01 cells/ml, the change was added to a buffer instead of being added to B_{ijk}. When the absolute value of the buffer exceeded 0.01 cells/ml, it was added to B_{ijk}. Thus, no fractional cells could enter the populations by mutation from, or recombination between, other strains, but the probabilistic meaning of rates of mutation and recombination was retained.

RESULTS

The critical results for the purpose of this paper are the times at which the triple mutant first appeared in each run, and the rates at which it ascended. I defined a measure, "R," as the log of the ratio of strain 111's density to the sum of the densities of all other strains. The figures show R as a function of time, and if selection were the only force causing strain 111's rise, the slope of such a graph at a given point would equal the natural log of the strain's fitness relative to an average fitness of one for all other strains present (HARTL 1980, p. 211; DYKHUIZEN and HARTL 1983). I calculated the "apparent selection advantage" as [exp(slope) − 1], with "slope" being the average slope over the first 40 hr after the strain arose, as a measure of rate of ascent. Note that "apparent selection advantage" may reflect the effects of mutation and recombination, as well as selection. Past studies (FISHER 1930; MULLER 1932, 1958, 1964; CROW and KIMURA 1965, 1969; MAYNARD SMITH 1968, 1971, 1978;

TABLE 1

Results for essential resources selection: number of hours required for strain (111) to arise

Mutation rate	χ = 0	χ = 10^{-16}	χ = 10^{-13}	χ = 10^{-10}
10^{-6}	733	733	732	496
10^{-8}	>40,000	>40,000	>40,000	10,858
10^{-10}	>40,000	>40,000	>40,000	>40,000

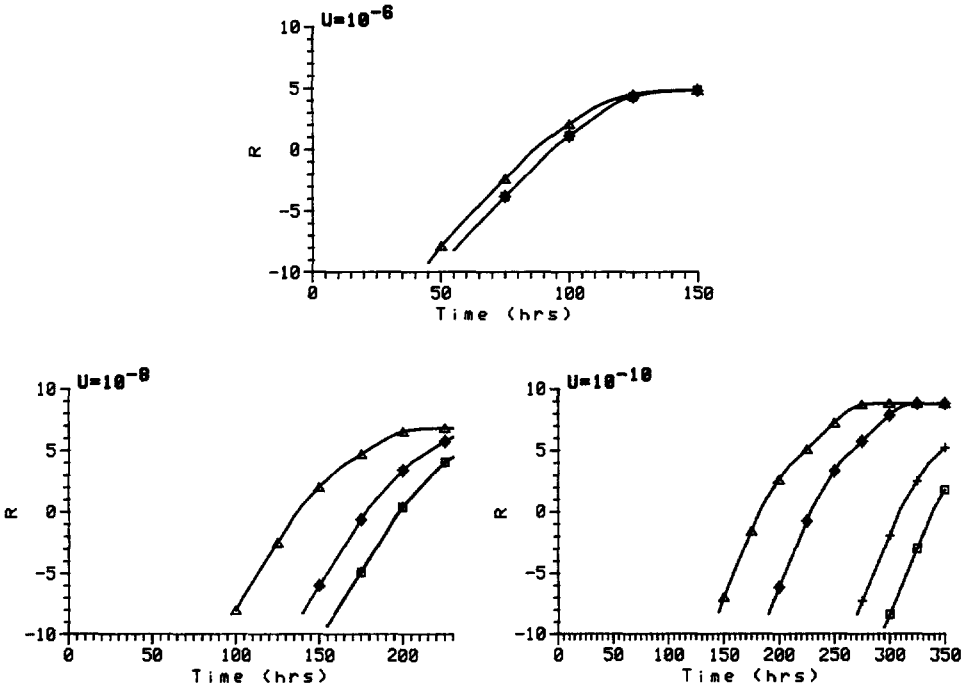

FIGURE 1.—Amino acids selection. □, $\chi = 0$; +, $\chi = 10^{-16}$; ◇, $\chi = 10^{-13}$; △, $\chi = 10^{-10}$. In all runs, glucose was supplied at 1000 μg/ml; all three amino acids were supplied at 20 μg/ml; and $\rho = 0.2$. R is defined in the text.

ESHEL and FELDMAN 1970; KARLIN 1973; BODMER 1970; FELSENSTEIN 1974) have generally interpreted earlier times of appearance and higher rates of ascent as higher rates of evolution, so that practice is continued here. The results for essential resources selection are presented in Table 1. The results for the other three cases are presented graphically in Figures 1–3, and Table 2 lists the apparent selection advantages for those cases.

Essential Resources Selection: Table 1 shows that recombination had no effect except when $\chi = 10^{-10}$. With $U = 10^{-6}$, this effect was considerable, advancing the appearance of the triple mutant by about one-third the time required without recombination. With $U = 10^{-8}$, $\chi = 10^{-10}$ clearly had a large effect, advancing time of appearance by at least two-thirds the time for no

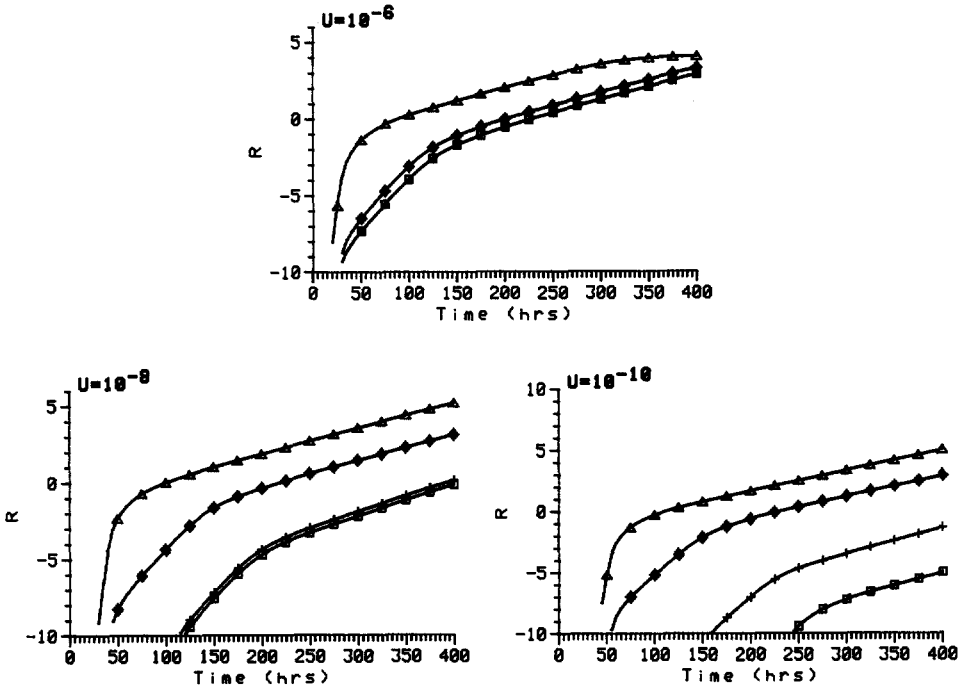

FIGURE 2.—Sugars selection. □, $\chi = 0$; +, $\chi = 10^{-16}$; ◇, $\chi = 10^{-13}$; △, $\chi = 10^{-10}$. In all runs, glucose and the other three sugars were supplied at 20 μg/ml, and $\rho = 0.2$. R is defined in the text.

recombination. Perhaps the most important result was that evolution was very slow, regardless of recombination.

I did not simulate the ascent of the triple mutant in this case, because (8) does not hold once the triple mutant has arisen, and using (1) and (12) directly would have resulted in enormous computing costs. Since the population densities of single and double mutants were very low, recombination would have made a negligible contribution to the ascent of a triple mutant strongly favored by selection.

Amino acids selection (Figure 1): Several important results are not shown in Figure 1. Under the initial conditions, each mutation increased a strain's fitness by a factor of about one-half. As evolution proceeded, and amino acid concentrations dropped, the fitnesses of the intermediate strains declined steadily and became more similar (*i.e.*, amino acid limitation became stronger). The fitness of the prototroph changed little, with its growth rate staying near maximum until it was well into its rise through the population.

At the highest mutation rate, 10^{-6} recombination had negligible effects. The highest recombination rate ($\chi = 10^{-10}$) caused the prototroph to arise only 5 hr earlier than it did without any recombination at all. At the intermediate mutation rate, 10^{-8}, the prototroph appeared 60 hr earlier with $\chi = 10^{-10}$ than it did with no recombination, but $\chi = 10^{-13}$ advanced its appearance only 15 hr. At the lowest mutation rate, the effects of recombination were more

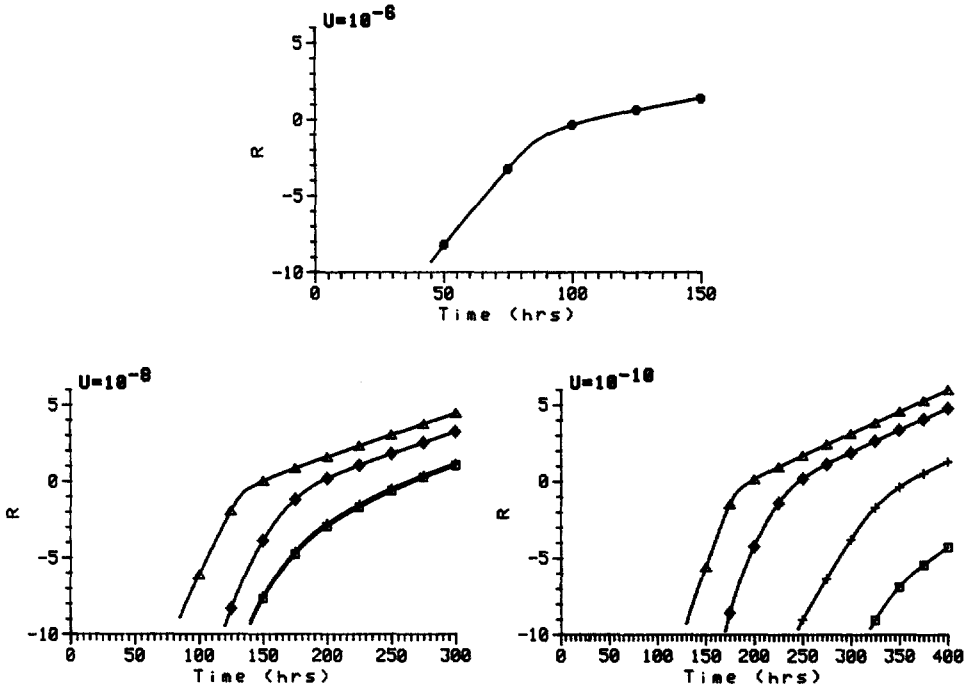

FIGURE 3.—Antibiotics selection. ✣, all values of χ; □, $\chi = 0$; +, $\chi = 10^{-16}$; ◇, $\chi = 10^{-13}$; △, $\chi = 10^{-10}$. In all runs, glucose was supplied at 20 μg/ml; the three antibiotics were supplied at 1.0 μg/ml; and $\rho = 0.2$. R is defined in the text.

TABLE 2

Apparent selection advantages

Selection scheme	χ = 0	10^{-16}	10^{-13}	10^{-10}
Amino acids				
all U	0.21	0.21	0.21	0.21
Sugars				
$U = 10^{-6}$	0.10	0.10	0.12	0.26
$U = 10^{-8}$	0.08	0.08	0.12	0.25
$U = 10^{-10}$	0.06	0.08	0.11	0.26
Antibiotics				
$U = 10^{-6}$	0.19	0.19	0.19	0.19
$U = 10^{-8}$	0.13	0.13	0.16	0.19
$U = 10^{-10}$	0.08	0.11	0.16	0.19

See DISCUSSION for explanation of "apparent selection advantages." These figures are averages for the first 40 hr after strain (111) arose, calculated from the curves in the figures.

dramatic. $\chi = 10^{-10}$ decreased time to appearance by 155 hr, $\chi = 10^{-13}$ by 110 hr and $\chi = 10^{-16}$ by 30 hr. Apparent selection advantages were almost unaffected by varying recombination or mutation rates.

Sugars selection (Figure 2): Initially, while the extra sugar concentrations were high, the single mutants had large advantages over wild type. Sugar concentrations dropped quickly, though, so the double mutants had much smaller advantages over the single mutants. By the time the triple mutant arose, sugar concentrations were usually quite low, so its selective advantage was modest.

When the mutation rate was 10^{-6}, recombination had practically no effect on the time at which strain 111 arose. Interestingly, however, the rates of rise were different for different gene transfer rate parameters. $\chi = 10^{-13}$ increased the rate of ascent marginally, $\chi = 10^{-10}$ increased the rate very dramatically for the first few tens of hours. Eventually, the rates of rise plateaued until all curves were nearly parallel, reflecting low resource levels generating only weak selection for strain 111. Recombination was more effective when the mutation rate was 10^{-8}. $\chi = 10^{-13}$ advanced the time of appearance by 70 hr and increased the apparent selection advantage by a factor of one-half. $\chi = 10^{-10}$ did not advance the time of appearance much more than $\chi = 10^{-13}$, but it more than doubled the rate of rise. It is clear that strain 111 became well established earlier with $\chi = 10^{-13}$ or 10^{-10} than without recombination. Recombination made dramatic differences at the lowest mutation rate. $\chi = 10^{-13}$ advanced the time of appearance 180 hr over $\chi = 0$, and $\chi = 10^{-10}$ did little more. At the end of 400 hr, strain 111 was vastly better established with $\chi = 10^{-13}$ or 10^{-10} than with no recombination. Recombination had nearly the same effects on apparent selection advantages as it did at $U = 10^{-8}$ and $U = 10^{-6}$. Varying the mutation rate changed apparent selection advantages only a little, whereas varying χ changed them substantially.

Antibiotics selection (Figure 3): Each mutation increased fitness by a factor of about one-half (virtually identical to the initial fitness relations in the amino acids case). Due to the lack of density dependence in relative fitness, however, the selective differences between strain 111, once it had appeared, and its predecessors were much less dramatic than under amino acids selection.

Recombination made no difference when the mutation rate was 10^{-6}. With the mutation rate at 10^{-8}, the curves separated somewhat. $\chi = 10^{-13}$ and $\chi = 10^{-10}$ advanced time of appearance slightly, and increased rate of rise slightly. At the lowest mutation rate, $\chi = 10^{-16}$ decreased time to appearance by 75 hr and $\chi = 10^{-13}$ by 150 hr, but $\chi = 10^{-10}$ by only 190 hr. Recombination had large effects on rates of rise, with apparent selection advantage at $\chi = 10^{-10}$ more than twice that without recombination. At $\chi = 10^{-13}$ or 10^{-10}, strain 111 clearly became well established much earlier than it did without recombination. Although recombination had only relatively small effects at the intermediate mutation rate, it had large effects at the lowest mutation rate.

DISCUSSION

The results show that recombination mediated by infectious gene transfer *could* affect rates of evolution in bacterial populations. The magnitude of the

effect depends on the relative rates of mutation and recombination and on the nature of selection. Recombination is most important when the rate of mutation is low, and niche expansion involves the acquisition of new abilities to use substitutable resources, such as sugars or other carbon sources. Recombination is the least effective when the mutation rate is high, and niche expansion occurs while the population is limited by several interactive-essential resources, such as certain amino acids or vitamins. At any given rate of mutation, the effects of recombination on the evolution of resistance to a set of growth inhibitors, such as antibiotics, are intermediate between those that occur under the sugars and amino acids selection schemes.

Effects of mutation and recombination rates: Increasing the mutation rate increased the rate at which cells of rare, or absent, genotypes were generated, so any effect of recombination on the time at which the triple mutant appeared was reduced. Losses due to mutation were always tiny, so increased rates of loss caused by increased mutation never slowed the growth of any strain. When recombination increased the rate at which the triple mutant ascended through the population, varying the mutation rate did not greatly change that effect of recombination. Recombination between abundant intermediate strains drove up the density of the triple mutant, even though the time at which it appeared was largely determined by the effects of mutation while the intermediate strains were rare. Increasing χ decreased the time required for the triple mutant to arise for the same reasons that increasing U did, except when the mutation rate was so high that the input into a strain due to recombination was always just a small fraction of the input due to mutation.

My result (which agrees with BODMER 1970) that the effect of recombination decreases with increasing mutation rate seems to contradict the result of other studies (CROW and KIMURA 1965; KIMURA and OHTA 1971; MAYNARD SMITH 1971; FELSENSTEIN 1974) that the effect of recombination increases with increasing rate of favorable mutations per genome per generation, but the two results can be reconciled. Increasing the per genome rate of favorable mutation in models considering a large number of loci increases the probability that new favorable single mutants will arise, so that recombination will have entirely new material to build into new genotypes. Increasing the single locus mutation rate in models that consider only a small number of loci increases the probability that mutation will generate double or triple mutants, but does not correspondingly increase the probability that a previously absent single mutant will arise (unless $U \ll 1/B_{222}$). This increases the effect of mutation without providing any new material for recombination to shuffle, so the effect of recombination relative to mutation is reduced.

Differences between selection schemes: The main objective of this study was to determine whether infectious gene transfer could affect rates of niche expansion in four well-defined sets of ecological circumstances. The results showed differences in the effects of gene transfer in the four cases, and to understand the differences we need to look at the specific ecological events that occurred in each case. R_{ijk} increases with the *square* of the densities of the strains recombining to generate strain ijk, whereas M_{ijk} increases simply with

the densities of the strains. Thus, R_{ijk} increases more rapidly with increasing densities of strain ijk's predecessors than M_{ijk}. But with U and χ set within the ranges I used, recombination between intermediate strains was weaker than mutation from those intermediates *when the intermediates were rare,* stronger than mutation *when the intermediates were abundant.* At low densities of intermediates, mutation was a stronger force generating new strains than recombination; at high densities of intermediates, recombination was stronger than mutation. The ecological details of the different selection schemes determined how fast densities of intermediates rose, and thus, determined how much effect recombination could have.

Under essential resources selection there was an adaptive "plain" to be crossed in reaching the triple mutant, so intermediates never reached densities high enough for recombination to be stronger than mutation. When $\chi = 10^{-10}$, recombination was strong enough to significantly augment mutation, and because both forces worked over very long time periods, recombination's small effect translated into a substantially reduced time to the appearance of the triple mutant. Obviously, an environment in which resource concentrations varied independently, in order to favor different single mutants at different times, could eliminate the adaptive "plain" considered here, speeding the evolution of independence from them all. Similarly, the adaptive plain would not occur in an environment where the essential resources were not equally limiting. This work cannot predict what effect recombination might have in such cases. Thus, the results in the case I considered are largely an artifact of choosing a particular set of environmental conditions that restrict evolution in a special way, but they may be interpreted as defining some effects of recombination in a case of neutral evolution.

In amino acids selection, amino acid concentrations dropped rapidly as the single mutants increased in density. This happened because the single and double mutants used amino acids more rapidly than did the wild type, as their growth rates were higher. Since the single mutants still required two amino acids, their selective advantages over wild type dropped with time. The same thing occurred with the double mutants—their limitation by the single amino acid they required became stronger with time. In sum, the competition between intermediates decreased all intermediates' fitnesses (and made their relative fitnesses density-dependent), so that their densities stayed relatively low, limiting the effects of recombination. The strengthening of amino acid limitation over time restrained recombination's effects.

Under the initial conditions, each mutation increased fitness by more under sugars selection than under amino acids selection (the first mutation increased per capita growth rate by a factor of 3 for sugars selection and by a factor of 1.5 for amino acids selection). Moreover, the sugars other than glucose were initially present in great excess, so it took many hours for those sugar concentrations to drop to levels that were limiting. Thus, relative fitnesses fell much less precipitously than under amino acids selection. The populations of intermediates rose relatively rapidly, so recombination had more effect than it did under amino acids selection. By the time that the triple mutant arose, however,

sugar concentrations were low enough to immediately limit its growth, making its selective advantage over the double and single mutants quite modest. Yet, the triple mutant ascended rapidly at first. Its rate of ascent increased with increasing χ, showing that input of cells by recombination between the other strains drove the early ascent. In contrast, under amino acids selection, the triple mutant was completely independent of the resources limiting the intermediates, so it rose very rapidly due to its large selective advantage. Recombination did not augment the rate of ascent in amino acids selection, primarily because selection on the triple mutant was strong, whereas intermediates had not reached densities high enough to cause high rates of recombination.

Antibiotic concentrations were constant, so selection for the intermediates did not drop with time under antibiotics selection. Since the effect of each additional mutation on fitness was roughly equal to the effect of a mutation at the beginning of amino acids selection, intermediates rose at rates higher than they did under amino acids selection. Thus, recombination had greater effects on the time at which the triple mutant appeared than under amino acids selection. The rate at which the triple mutant ascended was increased by increased χ values, for the same reasons as given for sugars selection. In this case, then, lack of density dependence in relative fitnesses led to greater effects of recombination than occurred under amino acids selection. Weaker selection for the intermediate strains kept recombination's effects more modest than under sugars selection.

In sugars and antibiotics selection, the effects of recombination on rates of ascent were large enough to substantially increase the probability that the triple mutant would become established in a population subject to effects of genetic drift, at least when rates of mutation were low. LENSKI and LEVIN (1985) have shown that the probability of a newly arising mutant becoming established in a chemostat-like environment, when the stochastic effects ignored by my models are taken into account, is directly proportional to that mutant's selective advantage, provided total population size is large. [See also FELLER (1957) for a more general treatment of the problem of "gambler's ruin", on which LENSKI and LEVIN's treatment is based.] MORAN (1962) and EWENS (1979) reached the same conclusion using more conventional population genetic models. Thus, Table 2 can be interpreted as showing that, under sugars selection, the triple mutant was 3 times more likely to become established with $\chi = 10^{-10}$, and 1.5–2 times as likely to become established with $\chi = 10^{-13}$, than with $\chi = 10^{-16}$ or no recombination (at $U = 10^{-8}$ or 10^{-10}). Under antibiotics selection with $U = 10^{-8}$, $\chi = 10^{-10}$ made the triple mutant about 1.5 times as likely to be established as with $\chi = 10^{-16}$ or 0. With $U = 10^{-10}$, $\chi = 10^{-10}$ or 10^{-13} doubled the probability that the triple mutant would not be lost. When conditions are like those of sugars or antibiotics selection, then infectious recombination at low rates might accelerate evolution by reducing the likelihood of early random loss.

Linkage disequilibrium and finite population size: The simulation runs always began with some linkage disequilibrium—single mutants were present at all but the lowest mutation rate, but no double or triple mutants were

initially present. The assumption that the culture vessel had a volume of 100 ml prevented the extremely low densities that double and triple mutants would have had under a mutation-selection balance. If single mutants were not present initially, they were generated by mutation without double or triple mutants occurring until much later. Thus, the genes favored by selection were strongly negatively associated when they first occurred. KARLIN's (1973) result that sexual recombination can accelerate evolution under such conditions was borne out here for infectious recombination.

Since my models were strictly deterministic, there were no effects of random sampling to cause genetic drift, and the Hill-Robertson effect could not occur. Thus, the effects described by FELSENSTEIN (1974) for finite populations did not occur. CROW and KIMURA (1965, 1969) considered the effects of prohibiting extremely low frequencies of genotypes, so that linkage disequilibrium occurred simply because of a limit on population size, as in my simulations. Thus, my "finite" population was not "finite" in FELSENSTEIN's sense, but was "finite" in the sense of CROW and KIMURA. Clearly my results agreed qualitatively with CROW and KIMURA's conclusion that recombination could accelerate evolution in finite populations.

Are the mutation and recombination rates that were used reasonable? Mutation rates for most bacterial loci, where mutation is observed as the loss of some function, are on the order of 10^{-6} or 10^{-7} mutations per cell division (LEWIN 1983). This would give a rate of around 10^{-6} mutations/locus/cell/hr in a chemostat running at a flow rate of 0.2. Mutations conferring new functions certainly occur at lower rates in many cases, so it is safe to take $U = 10^{-6}$ as an upper limit, with lower rates being more likely.

It is much harder to determine what values are reasonable for χ. SELANDER and LEVIN (1980) argued that χ must be 10^{-13} or lower in natural *E. coli* populations to be consistent with their finding of highly nonrandom associations of alleles at 20 loci studied electrophoretically. LEVIN (1981) subsequently argued that χ could be on the order of 10^{-13} or lower (but not much higher) for most conjugative plasmids of *E. coli*, based on known values for rates of plasmid transfer (LEVIN, STEWART and RICE 1979) and reasonable speculation on the likelihood of a plasmid carrying host genes with it as it transfers. More recent studies of *E. coli* population structure have reinforced the view that there is little gene exchange in natural populations (CAUGANT, LEVIN and SELANDER 1981; CAUGANT *et al.* 1983; OCHMAN *et al.* 1983; WHITTAM, OCHMAN and SELANDER 1983a,b). In particular, WHITTAM, OCHMAN and SELANDER (1983b) found no relationship between the level of linkage disequilibrium and map distance between 27 pairs of loci. If recombination occurred at a significant rate, a negative correlation would be expected. Moreover, surveys of natural populations of *E. coli* have found that strains incapable of gene transfer are common (LEDERBERG 1951; LEDERBERG, *et al.* 1951; LEDERBERG, CAVALLI and LEDERBERG 1952; ORSKOV and ORSKOV 1961a,b), but the techniques used in these early studies would not have detected some conjugative plasmids. Thus, I would argue that 10^{-10} represents an upper bound on χ, 10^{-13} is a

possibly reasonable value and 10^{-16} is a low, but reasonable, value for most *E. coli* strains.

A second argument supports this view. Estimates of χ for crosses of an F′ (lac^+ pro^+) strain (CSH 23; MILLER 1972) with various F^- strains in the laboratory generally give values on the order of 10^{-10} (S. HATTINGH and B. R. LEVIN, personal communication). F′ plasmids have recombination rates that are higher than those of most plasmids. Moreover, matings between a plasmid-bearing strain and a plasmid-free strain (as in the F′ × F^- matings) will give higher χ values than matings between two plasmid-bearing strains (as is postulated in my models) (LEWIN 1977). Thus, an upper bound on χ values in my models must almost certainly be 10^{-10}, at the highest. One must keep in mind, however, that such a limitation on χ does not mean that rates of recombination are always very low. If the two strains (a^+b^- and a^-b^+) undergoing gene exchange are at densities of 10^{-6} cells/ml and $\chi = 10^{-10}$, 100 a^+b^+ recombinants per milliliter will be produced each hour. Mutation with $U = 10^{-6}$ would produce only two cells of the recombinant genotype per milliliter per hour.

Given these estimates of χ, my results suggest that recombination is likely to be important only when niche expansion involves traits for which the mutation rate is low and when selection acts as it did under sugars or antibiotics selection. Other species of bacteria may have higher rates of gene transfer in nature, but few relevant data have been collected. In particular, Bacillus, Streptococcus, Haemophilus, and Neisseria species have efficient transformation mechanisms (SMITH and DANNER 1981), and thus, may have higher rates of gene transfer in nature.

One set of experiments on the evolutionary role of recombination in bacteria has been carried out. GRAHAM and ISTOCK (1978, 1979, 1981) cultured *Bacillus subtilis*, which undergoes transformation spontaneously, in sterilized soil. When they grew double auxotrophs with genotypes of the form $a^+b^+c^-d^-$ and $a^-b^-c^+d^+$ alone, the recombination $a^-b^-c^-d^-$ never occurred. When the two double auxotrophs were grown together, the quadruple auxotroph arose and eventually dominated many of the cultures. Thus, gene transfer seems to have made the evolution of the quadruple auxotroph possible. The single experiment that has been carried out suggested that gene transfer may affect bacterial evolution under realistic growth conditions. Only further experiments can assess the importance of the more complicated ecological factors that have been discussed here.

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APPENDIX

Let us assume that all strains other than 111 have the same growth rate and that strain 222 is initially at ecological equilibrium. Thus, all intermediate strains accumulate only by mutation and recombination. I also assume that no *111* cells arise while (4) to (7) are used to calculate growth rates and cell densities. This does not mean that strain 111's growth rate must be zero, however, because I allowed fractional cells to accumulate in a buffer until a whole cell was produced (see METHODS). Equations (4) to (7) were used to find the time required for the first whole 111 cell to be produced. The equations do not hold beyond that point, since strain 111 would quickly drive down the equilibrium densities of all other strains. The derivation of (4) is straightforward. From (1),

$$\frac{dB_{222}}{dt} = U(B_{122} + B_{212} + B_{221} - 3B_{222}) + \chi[B_{122}B_{2\cdot\cdot} + B_{212}B_{\cdot 2\cdot} + B_{221}B_{\cdot\cdot 2} - B_{222}(B_{1\cdot\cdot} + B_{\cdot 1\cdot} + B_{\cdot\cdot 1})].$$

Note that

$$B_{1\cdot\cdot} = B_{122} + B_{112} + B_{121} = B_S + 2B_D = B_{\cdot 1\cdot} = B_{\cdot\cdot 1}$$

$$B_{2\cdot\cdot} = B_{222} + B_{212} + B_{221} + B_{211} = B_{222} + 2B_S + B_D = B_{\cdot 2\cdot} = B_{\cdot\cdot 2},$$

where $B_S = B_{221} = B_{212} = B_{122}$ and $B_D = B_{112} = B_{121} = B_{211}$. Substituting B_S and B_D, where appropriate, gives

$$\frac{dB_{222}}{dt} = 3U(B_S - B_{222}) + 3\chi[B_S(2B_S + B_D) - 2B_{222}B_D]. \quad (4)$$

Consider one of the single mutants, strain 122:

$$\frac{dB_{122}}{dt} = U(B_{222} + B_{112} + B_{121} - 3B_{122}) + \chi[B_{222}B_{1\cdot\cdot} + B_{112}B_{\cdot 2\cdot} + B_{121}B_{\cdot\cdot 2} - B_{122}(B_{2\cdot\cdot} + B_{\cdot 1\cdot} B_{\cdot\cdot 1})].$$

Substituting in B_S and B_D as above gives the general equation,

$$\frac{dB_S}{dt} = U(B_{222} + 2B_D - 3B_S) + \chi[2B_D(2B_{222} + B_D) - B_S(4B_S + B_D)]. \quad (5)$$

Similarly, consider a double mutant, strain 112:

$$\frac{dB_{112}}{dt} = U(B_{212} + B_{122} - 3B_{112}) + \chi[B_{212}B_{1\cdot\cdot} + B_{122}B_{\cdot 1\cdot} - B_{112}(B_{2\cdot\cdot} + B_{\cdot 2\cdot} + B_{\cdot\cdot 1})].$$

Thus,

$$\frac{dB_D}{dt} = U(2B_S - 3B_D) + \chi[2B_S^2 - B_D(2B_{222} + B_S + 4B_D)]. \quad (6)$$

Finally, consider strain 111:

$$\frac{dB_{111}}{dt} = U(B_{112} + B_{121} + B_{211}) + \chi(B_{211}B_{1\cdot\cdot} + B_{121}B_{\cdot 1\cdot} + B_{112}B_{\cdot\cdot 1}).$$

Substituting in B_S and B_D yields

$$\frac{dB_{111}}{dt} = 3UB_D + \chi[3B_D(B_S + 2B_D)] \quad (7)$$